1015 The effects of different thymidine concentrations on DNA replication in cells synchronized by protracted FdUrd treatment. J.B. Schvartzman*, D.B. Krimer* and J. Van't Hof. Biology Department, Brookhaven National Laboratory, Upton, New York 11973. (Intr. by M.A. Bender).

Single-cell autoradiography, cytophotometry and velocity sedimentation in alkaline sucrose gradients were used to analyze the effects of different concentrations of thymidine (Thd) on DNA replication in FdUrd-synchronized cells of pea-root meristems. Thd concentration decisively influenced the rate of cell progression through the S phase. When cells were released from the G1-S block in the presence of $1x10^{-4}$M Thd DNA replication was completed in 4-5 hr. The S phase lasted 10-12 hr when the cells were grown in $1x10^{-5}$M Thd and DNA synthesis stopped after 20% of the genome was replicated when the cells were cultured with $1x10^{-6}$M Thd. Velocity sedimentation in alkaline sucrose gradients showed that the rate of fork movement was partially responsible for this effect. The experiments also showed that regardless of Thd concentration, nascent strands continually grew until a certain size was achieved. This size, however, varied according to Thd concentration. The weight average molecular weight of the accumulated nascent strands was $30x10^6$ when the cells were cultured with $1x10^{-4}$M Thd, $16x10^6$ when they were grown with $1x10^{-5}$M Thd, and $12x10^6$ when they were cultured with $1x10^{-6}$M Thd. It is possible that in higher plants DNA replication occurs as in SV40, where fork movement progresses stepwise and is temporarily halted at specific sites on the template. If this is the case, resumption of fork movement would depend on Thd concentration. However, since the growing of nascent strands ceases when two forks moving in opposite directions meet, it is also possible that the differences in size observed could be due to a change in the frequency of replicon initiation events. In this case, replicon size would vary according to Thd concentration. Finally, the possibility also exists that an excess of Thd enhances the ligation of completed neighboring replicons, in which case Thd concentration during chases should be carefully watched.

1016 Specific Peptide Synthesis During the Cell Cycle of CHO Cells. J.T. Westwood*, E.B. Wagenaar, and R.B. Church*, Department of Medical Biochemistry, The University of Calgary and Department of Biology, The University of Lethbridge, Alberta, Canada. (Intr. by J.J. Heikkila).

It has previously been demonstrated that protein synthesis is required for the initiation of mitosis in Chinese hamster ovary (CHO) cells. To determine whether or not there is a specific peptide or peptides synthesized just prior to mitosis in CHO cells the peptide patterns of several stages of the cell cycle were examined using two dimensional polyacrylamide gel electrophoresis (2-D PAGE). Cells were synchronized by either mitotic shake-off or a combination of shake-off and hydroxyurea treatments. Efficiency of the synchrony techniques was monitored by following the incorporation of ^{3}H-thymidine and analyzing individual cell DNA content using the Ortho system 50 fluorescent cell sorter. Early and late G_1, early and late S, mid and late G_2 as well as mitotic cells were labelled for 30 minutes with ^{35}S-methionine. Protein samples were run on both equilibrium (O'Farrell) and nonequilibrium pH gradient (NEPHGE) 2-D PAGE and autoradiograms made. Over 800 peptides can clearly be resolved on the autoradiograms and initial analysis indicates that there are several new peptides being synthesized in the transition from G_1 to S phase and from mid G_2 to mitosis. In addition, the synthesis of some peptides diminishes greatly between phases. These and other previous results indicate that there may be specific proteins involved in the initiation of mitosis in mammalian cells.
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1017 Oocyte Maturation: A Model System for Studying the Role of Early Ionic Events Involved in Reversal of the Quiescence. Jean B. Lum* and I. L. Cameron, Department of Anatomy, The University of Texas Health Science Center at San Antonio, S.A., Texas. (Intr. by V. Williams).

Oocytes, 1.2mm in diameter were removed from Xenopus laevis ovaries and exposed to progesterone (2.5 µg/ml for 30 min in Ringer's) to induce completion of the first maturation division or germinal vesicle breakdown (GVBD). Exposure of stimulated oocytes to amiloride, a drug known to inhibit passive influx of Na^+, at different times after progesterone addition revealed that an amiloride sensitive inhibition period developed at 15 min after progesterone and then disappeared at about 45 min after progesterone addition. Choline, Li^+ and Cs^+ but not NH_4^+ could be substituted for Na^+ in Ringer's and still permit GVBD. Oocyte maturation also occurred in sucrose made iso-osmotic to normal Ringer's and demonstrated amiloride sensitive inhibition. Oocytes incubated in Ca^{2+} free or K^+ free Ringer's underwent GVBD and developed an amiloride sensitive inhibition. However, when K^+ was substituted for Na^+ in Ringer's (i.e. high K^+), the oocytes were observed to undergo a more rapid GVBD after progesterone stimulation but did not develop an amiloride sensitive inhibition. Overall, these experiments show that an external source of ions is not required for GVBD and that the internal ion stores are sufficient to supply the oocyte's needs towards GVBD, the development of an amiloride sensitive inhibitory period seems to be dependent upon an initial surge of free Na^+ which may be related to the surge in free Ca^{2+} and finally that once an amiloride sensitive pathway develops it becomes integrated in the sequence of events leading to GVBD. In summary, these and other data in the literature are presented in a working hypothesis to clarify the role of early ionic events in progesterone stimulated oocyte maturation. (Supported by NSF Grant # PCM 8104084).

1018 The Intranuclear Concentration of Na but not Mg, K, P or S Correlates with Proliferative Activity in Rapidly Dividing Tumor and Normal Cells. I. L. Cameron and K. E. Hunter*, Department of Anatomy, The University of Texas Health Science Center at San Antonio, S.A., Tx.

Our past electron probe X-ray microanalysis data implicate the intracellular and the intranuclear concentration of Na as playing a role in both mitogenesis and overt neoplastic transformation. We are now doing studies in an attempt to more directly implicate Na as a causal factor in the control of cell proliferation in normal and tumor cells. In this experiment Ajax mice with a rapidly growing H6 hepatoma were randomly placed into two groups. The mice in one group were given three injections of amiloride, a drug reported to inhibit Na influx in rapidly dividing mammalian cells, made up in lactated Ringer's solution at a concentration of 1.0 µg/g body weight and each injection was spaced eight hours apart. The other group of mice were given the same series of injections of lactated Ringer's, but without amiloride. Both groups of mice were also given an injection of tritiated thymidine at a dose of 1 µ Ci/gm (sp. ac. 6.0 Ci/mM) body weight three hours after the last injection of amiloride or Ringer's solution and all mice were killed by decapitation one hour later at noon. Segments of the cortex of the tumors and the duodenum were then processed either for electron probe X-ray microanalysis or for autoradiography. We found that injections of the tumorous mice with amiloride significantly lowered the intranuclear Na concentration, but not the concentration of Mg, P, Cl or K and also significantly lowered the proliferation rate (labeling index) in tumor cells. This correlative observation between intranuclear Na and cell proliferation rate also held true for the rapidly dividing normal cell population of duodenal crypt enterocytes. That Na alone changed in parallel with cell proliferation activity strongly implicates the intracellular concentration of Na in the ionic regulation of cell proliferation. Supported by NSF Grant PCM-804084.