mediation (after proper discrimination), with glycine, L-alanine and L-serine. Under the same conditions, no substantial changes in transport activity were detected with L-leucine, L-phenylalanine and L-lysine, which are preferential substrates of transport systems L and Ly+ (Christensen, 1969). Discrimination from interfering agencies by transport-specific substrates indicated that the ASC system (Christensen, 1969) does not contribute to the stimulation of amino acid-transport activity by serum.

These results provide evidence that changes in transport activity for amino acids occur much earlier than the onset of DNA synthesis in cell populations induced by serum to enter the S-phase of the cell cycle. These changes consist of a cycloheximide-inhibitable increase in transport $V_{\text{max}}$ for amino acid substrates of the A-system mediation. Interestingly enough, the transport A-system appears to be the target mediation on which widely different (nutritional and hormonal) factors converge to control the amino acid-transport process (Guidotti et al., 1975, 1976; Robinson, 1976).

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Nuclear-Protein Changes in the First Cleavage Cycle in Fertilized Sea-Urchin Eggs

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Evidence is accumulating for structural differences between the histones of sperm and adult cells, especially for histone I. In echinoderm the switch from histone I to 1 occurs at gastrulation, but little is known about egg histones in early cleavage stages. This is primarily due to the difficulties inherent in obtaining good yields of pure nuclei when the nuclear/cytoplasmic ratio is low and a relatively large amount of extranuclear DNA and basic protein is present. Here nuclei were prepared by a simplification of earlier procedures (Loeb et al., 1969) and the phosphorylation of histone and non-histone proteins was followed during the first cleavage cycle.

Eggs were obtained in the usual way and the final preparations (up to 3 x 3 litres) contained 0.1–0.5 ml of packed eggs/10 ml of suspension. Pulse-labelling with $[^{32}\text{P}]P$, over several time periods in the first cycle was possible, since it was found that development could be arrested by the addition of 300 g of crushed ice at $-10^\circ\text{C}$/litre of suspension, which with stirring decreased the temperature to 0°C in less than 1 min. This treatment had no deleterious effect, and if the chilled eggs were restored to the original temperature development was resumed in the normal way, at least to hatching. Cleavage stages were followed microscopically.

*Echinus esculentus* eggs were sedimented for 5 min at 2000g and washed three times with sea water. The packed eggs were then mixed with 1.5 vol. of 2.5 M-sucrose/0.1% NaHSO$_3$ and homogenized in a Teflon–glass homogenizer. The material was then
centrifuged for 30min at 1400g to give white well-pelleted nuclei. Considerable time was saved by mixing the packed eggs with 2.5m-sucrose for homogenization, and nuclei from 9 litres of suspension could be obtained within 2h. The nuclei were extracted with 2.5ml of 2M-NaCl/0.1% NaHSO3/0.025m-borate buffer, pH 8.1/10ml of packed eggs by standing with occasional stirring for 1h before centrifugation for 30min at 26000g. To minimize proteolysis, equilibrium dialysis was avoided and the supernatant diluted directly with 0.1% NaHSO3 to give 0.15M-NaCl. After stirring for 1h the material was centrifuged (30 min at 26000g) to give 0.15M-NaCl-soluble non-histone proteins, and the pellet extracted twice with 250mm-HCl (1.0 and 0.5ml/10ml of packed eggs). The supernatant was dialysed against 0.1% NaHSO3 and clarified as before to give the acid-extractable nuclear proteins. All the operations were carried out at 0°C. Histones were isolated from the total acid-extractable proteins by standard methods and analysed (Ord & Stocken, 1975). The DNA content of the nucleoprotein pellet was determined (Burton, 1956).

The amount of protein soluble in 0.15M-NaCl greatly exceeded the amount extracted from the nucleoprotein pellet by 250mm-HCl and increased steadily from fertilization, reaching a peak in G2-phase (Fig. 1d).

From the volume of the packed eggs before homogenization the approximate number of eggs present could be calculated (Giudice, 1973). In the first cell cycle DNA is synthesized during fusion and through telophase. The amount of DNA in the nucleoprotein from fertilized eggs at stages from 50min (just after fusion) to early prophase (1h 45min) was 2.8±7pg/egg (Fig. 1f), which is consistent with the expected tetraploid amount of DNA based on Giudice's (1973) report of 0.8pg/haploid nucleus.

Analyses of the electrophoretically homogeneous basic protein analogous to mammalian histone 1 showed a high lysine and substantial arginine content similar to histone 1 of echinoderm spermatozoa (Palau et al., 1969). Histone 1 is not synthesized in early cleavage stages in echinoderms (Orengo & Hnilica, 1970), and since the proportions of histone 1 to DNA did not decrease by the first division (Fig. 1e) redistribution of existing histone 1 must occur. Weight ratios of histone 1/DNA for the unfertilized eggs and for times in the first cycle other than those immediately preceding DNA synthesis were similar to those in mammalian systems (0.25±0.04 histone 1/DNA).

Growth and division are associated with 32P uptake into histones and nuclear non-histone proteins in all eukaryotic systems so far examined [for references see Ord & Stocken (1975)]. This occurred also for the sea-urchins studied here; [32P]P1 was incorporated into the acid-soluble nuclear proteins during the first cleavage cycle (Fig. 1b and 1c), whereas uptake into unfertilized eggs was negligible. The extent of phosphorylation was given by the total amount of phosphate released by alkaline phos-

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Fig. 1. Cyclical changes in echinoderm nuclei through the first cleavage cycle

(a) Escherichia coli alkaline phosphatase-releasable P1 from acid-extractable proteins of Paracentrotus (●) and Echinus (○). The results from Paracentrotus are plotted on a time-scale equivalent to that in Echinus. (b) [32P]P1 incorporation into acid-extractable non-histone proteins from the 0.15M-NaCl-insoluble fraction of Echinus nuclei. [32P]P1 was present in the sea water for 20min. The ordinate gives the net radioactivity expressed as c.p.m. relative to the specific radioactivity of the [32P]P1 pool in the eggs. Radioactivity counts were made on each of the bands of the non-histone proteins separating by acid/urea/polyacrylamide-gel electrophoresis; 30µg of protein was applied to each gel. (c) [32P]P, uptake into histones. The results were obtained as for (b). The radioactivity associated with histones 1, 3 and 2A (●) was identical. †. Radioactivity migrating in the region of histone 2B through mitosis. (d) The ratio by weight of 0.15M-NaCl-soluble non-histone chromosomal proteins/nuclear DNA in Echinus. (e) The ratio by weight of histone 1/DNA in Echinus. (f) DNA content (pg/nucleus) in Echinus. The right-hand ordinate gives the ploidy.

1977
DNA Histone 1

- 0.15M-NaCl-soluble non-histone Net radioactivity relative to the [32P]P pool (c.p.m.) (nmol/mg of protein) (pg/nucleus) DNA ratio protein/nuclear DNA ratio

Ploidy

Fusion
Start of prophase
Furrow Cleavage

Graphs showing changes over time after fertilization:
- Graph (a): Phosphate released (nmol/mg of protein)
- Graph (b): Net radioactivity relative to the [32P]P pool (c.p.m.)
- Graph (c): Protein/nuclear DNA ratio
- Graph (d): Histone 1/DNA ratio
- Graph (e): DNA (pg/nucleus)
- Graph (f): Time after fertilization (min)

Ploidy levels:
- 1n
- 2n
- 4n
- 8n
Phosphatase from acid extracts from *Paracentrotus* and *Echinus* nuclei. Phosphorylation appeared to be promoted immediately after fertilization; a second peak occurred in prophase (Fig. 1a). $^{32}$P incorporation into non-histone proteins was greater than that into the histones (Fig. 1c). Uptake of $^{32}$P represents the net result of protein kinase and phosphatase activities. Immediately after fertilization the kinase becomes active, probably owing to the release of cyclic AMP (see Giudice, 1973); after fusion protein phosphatase appears dominant. A similar cycle follows through prophase and telophase.

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**Nucleosomes and Deoxyribonucleic Acid Methylation**

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In eukaryotes the only postsynthetic modification to occur to DNA is the methylation of certain cytosine residues. Although 2–5% of cytosines are methylated (Kappler, 1971) nothing is known for certain about the function of these methyl groups. The evidence (Dosocoil & Sorm, 1961) suggests that most, if not all, of the methylated cytosines occur in the sequence MeCpG, but the distribution of this methylated dinucleotide along the DNA molecule is not known.

The DNA in eukaryotic cells is associated with histones to form nucleosomes (Oudet et al., 1975). A length of DNA comprising about 200 nucleotides is present in each nucleosome apparently wrapped around a core of eight histone molecules (Thomas & Kornberg, 1975). Treatment of chromatin or nuclei with micrococcal nuclease first cuts the DNA between the nucleosomes, releasing 200 nucleotide-long fragments, and then trims back the ends of these fragments to yield a 140 nucleotide-long product (Morris, 1976).

There has been some controversy as to whether the histones are fixed to a particular stretch of DNA or whether they are free to slide or roll along the DNA molecule (Steinmetz et al., 1975; Cantor, 1976). If the latter is the case the base composition of the DNA within a nucleosome (i.e. the 140 nucleotide-long fragment) would be expected to show no deviation from that of the total DNA. However, in the former case it is possible that some particular base sequence may form a signal for the attachment of the histones. It is conceivable that methylcytosine could form part of this signal and indeed its frequency of occurrence (one per 100–250 nucleotides) is compatible with this idea.

We have investigated the distribution of 5-methylcytosine among the nuclease digestion products from nuclei of Chinese-hamster ovary (CHO) cells previously incubated with $[1^{14}$C]deoxycytidine (The Radiochemical Centre, Amersham, Bucks., U.K.). After nuclease digestion the nuclei were dissolved in a solution containing 1% sodium dodecyl sulphate, and the protein was removed by phenol extraction (Adams, 1974). The DNA was then fractionated on 1.5% agarose gels (Tegtmeyer, 1972), stained with ethidium bromide, and the bands were cut out. The DNA was eluted with 0.3m-NaOH, precipitated and washed with 0.5m-HClO$_4$ and hydrolysed with 12m-HClO$_4$. The bases were separated by chromatography (Adams, 1974), and the degree of methylation was...