Srivastava[8] have earlier shown that di- and polyamines such as agmatine, putrescine, cadaverine, spermidine and spermine significantly reduced pigment efflux from beet root discs, by reducing membrane permeability, and suggested that this stabilizing effect on beet root membrane is due to their binding to phospholipid components of the membrane. The case with amino acids may be similar. Proline has been shown to act as a cryoprotectant in maize and potato protoplast cultures by stabilizing the membrane; besides proline, γ-amino butyric acid, hydroxyproline and aspartate also helped in cryoprotection of the maize protoplast[9]. Altman et al.[10], studying stabilization of oat leaf protoplasts, also showed that L-arginine and L-lysine, besides other polyamines, inhibited their senescence by stabilizing the protoplast membrane.

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Biochemical studies of CELO virus: an oncogenic avian adenovirus[1]

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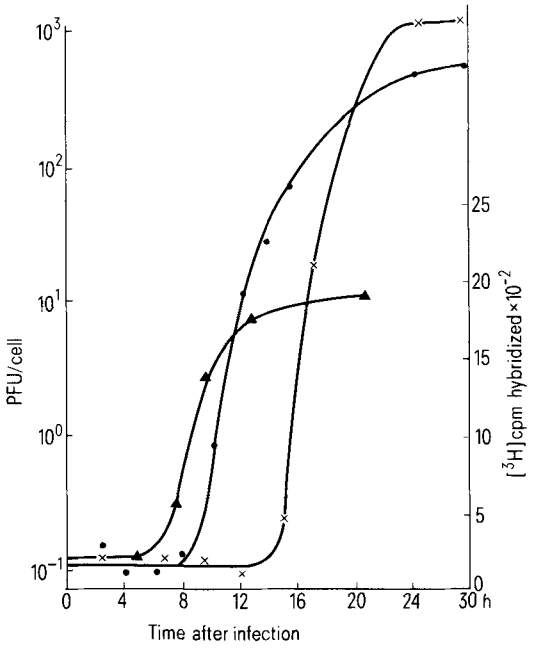
Summary. The onset of viral mRNA and DNA synthesis in CELO virus infected cells occurred about 5 and 8 h respectively after infection. Viral maturation occurred approximately 6 h after DNA synthesis at 14 h post infection.

Chick embryo lethal orphan (CELO) virus is a DNA containing avian adenovirus[2,3]. Its oncogenic potential was established by inoculation of CELO virus into non-permissive hosts[4,5] and in tissue culture cells[6,7]. Moreover, fragment mapping of restriction enzyme digests of CELO virus DNA[8] and sequences of viral DNA found in transformed cells[9] have been reported. However, the biochemical events associated with macromolecular syntheses of the viral multiplication cycle have not been published. The events reported here are concerned with the time of viral mRNA and DNA synthesis and the time of viral maturation occurring in chick embryo kidney tissue culture cells infected with CELO virus.

DNA-RNA hybridization was employed to determine the time of viral mRNA synthesis. CELO virus was inoculated into embryonated 11-day-old chicken eggs and the allantoic fluid was harvested at time of embryo death. The virus was purified by isopycnic CsCl gradient centrifugation[2,10], and DNA was extracted from the virus by digestion with pronase B (100 µg/ml) and 1% sodium dodecyl sulfate for 30 min followed by purification with a phenol, chloroform, and isoamyl alcohol mix (25:24:1)[2,11]. After denaturation, DNA was immobilized onto 6.5-mm nitrocellulose filters (1 µg/filter)[12]. Cells for tissue culture and virus infection were obtained from the kidneys of 19-day embryos. They were minced and trypsinized (0.1% trypsin and 0.05% EDTA). Cells were grown in MEM Eagles with Earle's salts supplemented with 10% tryptose phosphate broth (TPB) and 10% calf serum (CS). Monolayers were established after 48 h of growth and used for CELO virus infection at 5 PFU/cell (see below). At various times after infection, up



Macromolecular events during CELO virus infection of chick embryo kidney tissue culture cells. Time of viral mRNA synthesis (▲—▲), viral DNA replication (●—●) and viral maturation (×—×) are indicated at various times after infection. Viral mRNA synthesis was determined by DNA-RNA hybridization and viral DNA and viral maturation were determined by plaque titration.

to 24 h, ^{3}H-uridine (50 Ci/mM) was added to the cells for 2 h and RNA for hybridization was phenol extracted from these calls[13]. Input counts of 50,000 cpm were added to the filters under non-saturating conditions[14]. As shown in the figure, viral mRNA synthesis begins at approximately 5 h post infection (p.i.).

The time of viral DNA synthesis was determined by the plaque assay method and the use of cytosine arabinoside (ara-C). The overlay medium for the plaque assay was an equal volume of 2×(MEM/TPB/CS) and 1.8% agar containing 0.0125% neutral red. Ara-C was first added to infected cells to determine the concentration needed for viral DNA inhibition. The table indicates that at a concentration of 1×10^{-5} M ara-C, viral DNA synthesis was inhibited by 0.0069% of non-treated infected cells.

At various times after infection, ara-C, at the inhibitory concentration, was added to CELO virus infected cells (5 PFU/cell). At 30 h p.i., all samples were harvested and plaque assayed. The strategy of this experiment was to inhibit any viral DNA synthesis after ara-C addition; however, any DNA synthesized prior to ara-C addition would proceed in forming infectious viral particles which could be plaque assayed and thereby indirectly indicate that viral DNA synthesis had occurred. The figure indicates that viral DNA synthesis begins approximately 8 h p.i.

The time of viral maturation was determined by infecting cell monolayers at 5 PFU/cell, harvesting the cells at various times and plaque titrating for infectious viral particles. As seen in the figure, CELO virus maturation occurred at approximately 14 h p.i., 6 h after the onset of viral DNA synthesis.

The sequence of events during CELO virus infection in chick embryo kidney tissue culture cells is as follows: viral mRNA synthesis precedes viral DNA synthesis by approximately 3 h. Presumably this early period is involved in the synthesis of nonviral protein molecules. After DNA synthesis, viral structural proteins are translated from late viral mRNA molecules as was shown in human adenovirus type 2[14]. It seems probable that similar events are also occurring in CELO virus infected cells. Viral maturation subsequently follows with the formation of infectious viral particles.

Determination of the molar inhibitory concentration of cytosine arabinoside to inhibit CELO virus DNA synthesis in chick embryo kidney cells infected at a multiplicity of 5 PFU/cell

Concentration of Ara-C	PFU/cell	% of control
10^{-3} M	0.01	0.0014
10^{-4} M	0.014	0.0020
10^{-5} M	0.049	0.0069
10^{-6} M	189	26.6
0 (control)	710	-

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Alkylation of protein by Tris-(2-chloroethyl)amine at the peptide bond[1]

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Summary. Tris-(2-chloroethyl)amine (TCEA), a strong alkylating agent, not only alkylates protein at the hitherto accepted reactive sites but also at the nitrogen of the peptide bond; this is, however, no hindrance for proteolysis.

Fingerprinting of a tryptic hydrolysate of purified human hemoglobin which had reacted with ^{14}C-tris(2-chloroethyl)-amine (TCEA) in vitro showed both ninhydrin positive spots lacking any radioactivity, and radioactive spots without a ninhydrin reaction[2]. Similar results were obtained when tryptically hydrolyzed β-chains of human hemoglobin which had been alkylated with ^{14}C-TCEA were chromatographed on DOWEX 1x2. Many of the radioactive peaks did not match the ninhydrin positive peaks[3].

A free NH_2-group (terminal or in ω-position of a basic amino acid constituent) is necessary in a protein or peptide for its chromogenic reaction with ninhydrin[4]. From the above observations it was concluded that alkylated hemoglobin - at least in part - did not yield free NH_2-groups but alkylated NH-groups at the N-terminal of the tryptically liberated peptides. Thus, hemoglobin must have been alkylated at the nitrogen of the peptide bond. An alkylated NH-group, however, is no longer accessible to the ninhydrin reaction[4]. In order to prove this assumption, the reaction has been studied using a synthetic peptide.

The artificial tri-peptide, glycyl-leucyl-tyrosine (G-L-Y) was alkylated by incubation with ^{14}C-TCEA in 0.2 M triethanolamine buffer, pH 7.0, at 37 °C for 60 min; molar ratio TCEA: peptide = 5. Theoretically, the highest specific