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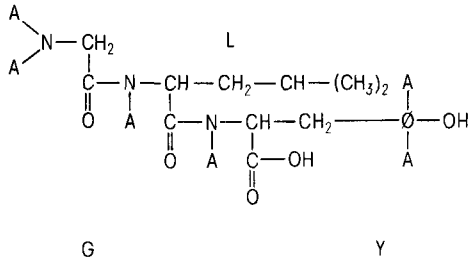
Summary. Tris-(2-chloroethyl)amine (TCEA), a strong alkylating agent, not only alkylates protein at the hitherto accepted reactive sites but also at the nitrogen of the peptide bond; this is, however, no hindrance for proteolysis.

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

activity possible would be 6 moles TCEA per mole peptide ('A' standing for alkylans):



Alkylation products were characterized by radioactivity, by absorption at 280 nm, and by spot tests[4,5]. Besides monoalkylation, cyclization, and/or cross-linkage of the peptide molecule(s) with the trifunctional alkylans can occur. (Alternatively, alkylation of Y at the phenylic OH-group cannot be excluded.)

Chromatography. When the incubation mixture was chromatographed on silica gel TLC plates spots of radioactivity were found which did not give a ninhydrin reaction[2] but were clearly visible under UV-light. Some alkylation products were isolated from the incubation mixture by gel filtration on a column of BIOGEL P-2 (1 × 145 cm; in 0.1 M NH_4HCO_3). After determining radioactivity and absorption at 280 nm in the eluted peaks sp. act. of 1, 1.5, 2, and 4 moles TCEA bound to 1 mole of peptide were calculated. Only products with specific activities less than 2 showed a slight color after treatment with tert-butylhypochlorite and starch iodine solution using the method of Mazur et al.[5].

Hydrolysis and amino acid analysis. As judged from the TL chromatograms of the original peptide and of the incubation mixture no splitting of the peptide into G, L, and Y, or G and L-Y, or G-L and Y occurred during alkylation and incubation, respectively. Free (alkylated) amino acids were obtained, however, by hydrolysis with 6 N HCl (under N_2 at 110 °C for 22 h) or with 6 N NaOH (under N_2 at 110 °C for 22 h) or with carboxypeptidase A (twice washed CP-A in 0.2 M $NaHCO_3$, pH 9.3 at 37 °C for 22 h). From a hydrolyzed alkylated peptide with a sp.act. of 1.5 (TCEA:peptide) L and Y could easily be detected with an automatic amino acid analyzer. G, however, gave no or only a very poor ninhydrin reaction. The amount of ninhydrin positive G, L, and Y decreased with increasing specific activity of the hydrolyzed peptide. None of the ninhydrin positive amino acid peaks, eluted from the ion exchanger of the amino acid analyzer, contained any radioactivity. The radioactivity was eluted with a LiOH solution which is generally used for regeneration of the column. When this eluate was filtered on a column of BIOGEL P-2, up to 6 radioactive peaks could be distinguished.

Autoradiography. TLC of the above hydrolysates of alkylated peptides did not yield a ninhydrin positive G spot. The higher the specific activity of the hydrolyzed peptide, the less was the color of the corresponding spot for G, L, and Y on the TL plate after development with ninhydrin. Autoradiography, however, clearly yielded radioactive spots on the TL chromatogram for all 3 amino acids. Their R_f-values were slightly different from those of free (not alkylated) G, L, and Y. Free G, L, or Y were not readily alkylated by TCEA under same conditions as described for the peptide. Although this is not explicable an even better accessibility for alkylation of the amino acid and/or of the peptide bond in the peptic compound may be indicated. Perhaps a more distinct polarity in the tripeptide favors a better reactivity of the terminal NH_2-group with the alkylans.

Ross[6] did not mention a reaction of protein, peptides or even Y with TCEA. Our results could be interpreted by suggesting that TCEA alkylates protein (and peptides) at the nitrogen of a free NH_2 group and/or at the nitrogen of the peptide bond as well. The unspecific ability of protein, previously treated with p-chloromercuribenzoate, N-ethylmaleimide, or diethylpyrocarbonate[2], to bind TCEA might support this idea. The lack of ninhydrin reaction and the failure to detect a H-N-bond[5] in the alkylated peptide seems to be due to its alkylation. The accessibility of a peptide bond to the alkylans TCEA obviously depends on the steric structure of the protein molecule.

The binding of the alkylans TCEA to the protein seems to be stronger than the peptide bond as judged from the hydrolysis experiment (with HCl or NaOH). Furthermore, the alkylation of the peptide bond seems to be no steric restriction for the enzymatic cleavage of the peptide with carboxypeptidase A or trypsin[2]. This fact might be an advantage for alkylation repair in the living cell.

1 This work was done in the Pharmakologisches Institut der Universität München, Nussbaumstr. 26, D-8000 München 2.

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Detection of factor VIII related antigens in long term cultures of rat endothelial cells [1]

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Summary. Rat endothelial cells in culture can be distinguished from fibroblasts and epithelial cells by their reaction with antisera against human factor VIII (AHF) associated proteins.

Endothelial cells can be identified in vivo and in culture by their morphological characteristics and by their ability to synthesize angiotensin converting enzyme[2-4] and antihemophilic factor VIII[5,6]. We have previously reported[7] the establishment of a strain of cells derived from a single cell cloned by the microplate method from a line of mixed