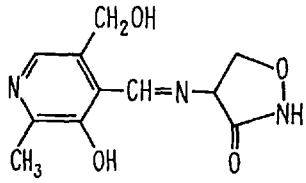
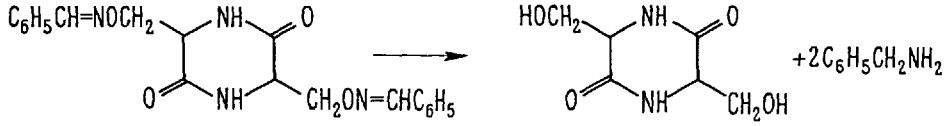


Unsere eigenen Untersuchungen brachten uns zur Vermutung, dass Cycloserin auf bis jetzt von uns nicht völlig geklärte Weise teils über DKP hinaus seine biologische Aktivität entfaltet. Die interessanten pharmakologischen Eigenschaften des DKP bieten eine gewisse Stütze für unsere Anschauungen über die Wirkungsweise von Cycloserin.



Es wurden vergleichende Untersuchungen über die antituberkulose Wirksamkeit von *D,L*-Cycloserin und *D,L*-2,5-Bis-(aminooxymethyl)-3,6-diketopiperazin *in vitro* an einer 12 Tage alten Kultur des *Mycobacterium tuberculosis* var. hominis $H_{37}Rv$ in flüssigem Nährmedium nach Proskauer-Beck durchgeführt. Die verwendeten Präparate wiesen Konzentrationen von 0,78 γ/ml bis 100 γ/ml auf. Nach 14tägiger Inkubationszeit bei 37° wurden die Ergebnisse makroskopisch abgelesen. Die vollständige Wachstumshemmung des *Mycobacterium tuberculosis* bewegte sich bei beiden Präparaten im Konzentrationsbereich von 6,25 bis 12,5 γ/ml. Die *in vivo* durchgeführten Orientierungsversuche brachten ebenfalls günstige Resultate.

Die Toxizität des *D,L*-2,5-Bis-(aminooxymethyl)-3,6-diketopiperazins ist bei peroraler Verabreichung gleich gross wie die des *D,L*-Cycloserins (DL_{50} 4 g/kg). Die nach der Applikation des *D,L*-2,5-Bis-(aminooxymethyl)-3,6-diketopiperazins beobachteten toxischen Symptome sind durch starke Niedergeschlagenheit gekennzeichnet, welche von Ptosis und Katalepsie begleitet wird. Jedoch ruft jede von aussen kommende Anregung starke Reizbarkeit hervor. Später treten Atmungsschwierigkeiten und Cyanosis hinzu. Nach Verabreichung von *D,L*-Cycloserin wurden praktisch dieselben toxischen Symptome beobachtet.

Im Gegensatz zum *D,L*-Cycloserin, das die Thiopenthalnarkose bei Mäusen bedeutend verlängert, zeigte sich *D,L*-2,5-Bis-(aminooxymethyl)-3,6-diketopiperazin in dieser Hinsicht ohne Einfluss. Die Resultate der vorläufigen pharmakologischen Untersuchungen deuten auch in anderen Richtungen auf die unterschiedlichen Eigenschaften der beiden Präparate hin (zum Beispiel in der Wirkung auf die krampferregenden Stoffe). Das 2,5-Bis-(aminooxymethyl)-3,6-diketopiperazin sowie seine Derivate sind Gegenstand weiterer pharmakologischer Untersuchungen.

Summary. It has been ascertained that racemic 2,5-bis-(aminooxymethyl)-3,6-diketopiperazine, being a transformation product of *D,L*-cycloserine, possesses the same effect against *Mycobacterium tuberculosis* as *D,L*-cycloserine itself.

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Uptake by Mammalian Cells of Nucleic Acids Combined with a Basic Protein

When nucleoproteins are dissociated with different techniques, nucleic acids apparently retain the whole genetic information[1,2] and protein moiety most of the immunological properties of the original complex. In addition, nucleoproteins have some biochemical and biological characteristics which are not displayed by their components: a remarkable resistance toward hydrolysis with enzymes for instance. The host-specificity and high efficiency of infection that whole viruses (but not the extracted V-DNA and V-RNA) possess, and the ability of ribosomes to serve as template for protein synthesis[3] (a property which is not shared by R-RNA) are other characteristics of intact nucleoprotein particles only.

We have been interested in investigating the properties of artificial ribonucleoprotein complexes deriving from the *in vitro* combination of a basic protein with nucleic acids of different types and sources, with the aim of using them as biochemical tools for a study of more complex systems such as viruses and ribosomes.

Experimental. Basic protein (MA) was prepared by methylation with methanol and HCl of bovine serum albumin (fraction V, Armour Labs.)[4–6]. Nucleic acids were obtained from mammalian cells and viruses by extraction with cold phenol[7,8]. HeLa cells, homogenously labelled by suspension culture in modified Hank's medium containing P^{32}-orthophosphate, and suspended in 0.1 *M* NaCl buffered at pH 7.1 with 0.1 *M* phosphates, were extracted with 2% duponol and water-saturated phenol[7,8]. Protein-free nucleic acids, precipitated with ethanol (95%, 6 vol, 18 h, 3°C) and redissolved in buffered saline (0.1 *M* NaCl, pH 7.1) were fractionated with 1.5 *M* NaCl (18 h, 3°C) into a sediment containing most of the high molecular weight

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ribonucleic acid (HMW-RNA) and a supernatant yielding most of DNA and low molecular weight ribonucleic acid (LMW-RNA)[7]. In some experiments the last fraction was used as such, for others it was treated with KOH (0.3 *M*, 18 h, 37°C) or nucleases, and subsequently neutralized and dissalted, or extracted with duponol and phenol, to obtain separate fractions of DNA and LMW-RNA. Concentrated suspensions of poliomyelitis virus were obtained by short sonication of cells cultured in suspension and harvested at the 8th hour after infection with multiplicity 5. Titration of poliovirus was carried out on monolayers of monkey kidney cells[9]. Columns employed for chromatographic separation of nucleic acids (PCS) consisted of several layers of supercel coated with MA[6-8].

Results. (a) When a protein-free extract of P^{32}-labelled nucleic acids from HeLa cells is loaded on a PCS-column and eluted with a salt gradient of increasing molarity 0.3 → 1.5 *M* NaCl constantly buffered at pH 6.8 with 0.05 *M* phosphates, four peaks of radioactive and UV-absorbing materials are obtained[7,8]. The first fraction washed out by 0.3 *M* NaCl contains nucleotides, bases, inorganic phosphates and other P^{32}-labelled or UV-absorbing molecules, the second peak is composed of LMW-RNA eluting at 0.5 *M* and having a $S^{\circ}_{20w} = 4$ s (M.W. = 2.5×10^4), and the third fraction is referred to DNA ($S^{\circ}_{20w} = 38$ s; M.W. = 3.2×10^6). The fourth peak (which can be further resolved by suitable gradients) contains two types of HMW-RNA eluting between 0.75 and 1.0 *M*, and having sedimentation coefficients of 16 s (M.W. = 0.6×10^6) and 27 s (M.W. = 1.2×10^6)[10], values quite similar to those reported for analogous nucleic acids from other cells. We may then conclude that MA combines reversibly with nucleic acids releasing them at different molarities which are characteristic for the bound molecules, and that nucleic acids elute from a PCS column without any apparent alteration of their biophysical properties. Ratio of nucleic acid phosphorus in exponentially growing HeLa cells was found to be the following LMW-RNA : DNA : HMW-RNA = 8 : 32 : 60.

(b) In a previous work[11] it has been shown that combination of MA with cellular nucleic acids of different origins results in their protection from the hydrolytic action of crystalline nucleases, *in vitro*. Do these combinations withstand also the intracellular enzymes when MA-bound nucleic acids are taken up by mammalian cells? Problem was analyzed by incubating with MA *in vitro*, P^{32}-labelled nucleic acids isolated from suspension cultures of HeLa cells[7,8]. Mixtures were subsequently incubated for a short while with monolayers of HeLa cells. After removal of unabsorbed radioactivity, monolayers were covered with unlabelled medium and allowed to multiply for two generations, in order to permit the penetration of labelled macromolecules inside the cellular body and expose them to the hydrolytic action of intracellular enzymes. P^{32}-labelled nucleic acids recovered by phenol extraction of the cell suspensions were analyzed by column chromatography, and results summarized in the Table. It can been seen that: (1) Mammalian cells take up far more protein-bound nucleic acids than protein-free molecules (increase in the uptake in different experiments was of the order of 40 times for DNA and HMW-RNA and 20 for LMW-RNA). (2) Intracellular nucleases hydrolyze nucleic acids that cells take up, while products of hydrolysis seem to be utilized for synthesis of new nucleic acids. (3) Apparently HeLa cells are unable to split down the complexes methylated albumin-nucleic acids within a two generation period.

(c) Problem whether nucleic acids absorbed by cells in combination with methylated albumin, still retain their biological properties, e.g. as carriers of new genetic informations inside the cellular body, was investigated with the use of nucleic acids from viruses which can replicate on HeLa cells. We have observed that infectious V-RNA obtained with cold phenol extraction of concentrated suspensions of poliovirus combines with methylated albumin and forms a complex which is taken up by HeLa cells but is not infectious. Since we have previously reported[8] that the complex of V-RNA with MA can be purified by column chromatography and then dissociated with 0.8 *M* NaCl, yielding free V-RNA still infectious for HeLa cells monolayers, we can then suppose that complex itself is stable to intracellular enzymes, which causes its components to be biologically inactive. Problem was further analyzed by injecting into the allantoic cavity of 8-day old embryonated eggs the mixture of methylated protein and V-RNA from poliovirus. This system has been studied in our Institute and proved to be a particularly useful tool for work with V-RNA, because polio V-RNA, but not

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Experimental conditions: HeLa cells homogenously labelled through growth for 3 generations in P^{32}-medium. Labelled nucleic acids extracted with cold phenol, precipitated by ethanol, fractionated by NaCl 1.5 *M*, de-salted and allowed to combine or not with methylated protein at 37°C. Aliquots of labelled nucleic acids or nucleoproteins incubated for 1 h at 37°C on monolayers of HeLa cells: excess removed by repeated washings. Monolayers covered with unlabelled media and incubated for 48 h at 37°C. Cells dispersed with trypsin, then centrifuged and washed five times, suspended in 0.1 *M* NaCl 0.1 *M* phosphate buffer pH 7.1, counted and extracted at 3°, 37°, 3°C with phenol and 2% duponol. Aliquots of the original nucleic acids solutions (controls) and of extracted cells fractionated by column chromatography. Radioactivity and O.D. at 253 mμ continuously recorded. Schmidt-Thannhäuser procedure carried out simultaneously as control. Efficiency of extraction judged from the UV-absorption values and the number of cells. In parenthesis percent distribution of recovered radioactivity among the three nucleic acid fractions obtained by partition chromatography.

Uptake and conservation of labelled nucleic acids in HeLa cells

Nucleic acid fraction	Methylated albumin	Incubation on HeLa monolayers	Recovered P^{32} nucleic acids after column chromatography (cpm/chrom. peak) S-RNA	DNA	R-RNA
S-RNA + DNA	—	—	1.1×10^6 (16%)	5.5×10^6 (79%)	3.3×10^5 (5%)
	—	+	5.7×10^4 (33%)	5.02×10^4 (29%)	6.5×10^4 (38%)
	+	+	5.8×10^5 (18%)	2.3×10^6 (76%)	1.5×10^5 (5%)
R-RNA	—	—	1.3×10^5 (2%)	3.7×10^5 (5%)	7.1×10^6 (93%)
	—	+	1.0×10^4 (19%)	1.8×10^4 (33%)	2.6×10^4 (48%)
	+	+	7.7×10^4 (4%)	2.6×10^5 (13%)	1.6×10^6 (83%)

intact viral particles, may replicate and induce a one-cycle production when injected into such embryonated eggs. In this system also, the combination of MA and V-RNA showed to be non-infectious over a 24 h period. If combinations of V-RNA with MA remain unaltered intracellularly, then one should expect to be able to block multiplication of viruses by giving MA to HeLa cells before infection with viral particles and their nucleic acids. Experiments carried out with vaccinia (virus containing V-DNA) and poliovirus or its V-RNA component, however, were unsuccessful, because a reduced yield of particles per cell was observed but conditions necessary to shun viral replication completely have not yet been met. Toxicity of MA for HeLa makes it difficult to saturate cells with basic protein in such way that single nucleic acid molecules could be conveniently trapped before they can transfer their genetic message to the cell.

Discussion. (a) Since we have previously found[7] LMW-RNA in both the washed pellet and the supernatant of a 105000 g centrifugation, our LMW-RNA chromatography fraction is not exactly the same as transfer ribonucleic acid (S-RNA) isolated by ultracentrifugation of cellular homogenates; the same distinction must be made for HMW-RNA and R-RNA from ribosomes. Our previous[11] and present data indicate that both these ribonucleic acids, as well as cellular DNA, when combined with MA, are stable toward the hydrolytic action of nucleases, *in vitro* and *in vivo*. Such a resistance seems due to a real combination and not merely to inhibition by MA of enzyme activity, because no competition was observed in the present of an excess of ribonuclease[11].

(b) The observed uptake of naked DNA by HeLa cells confirms the reports from several Laboratories[12–16] which have recently described the penetration of homologous and heterologous DNA molecules into the nuclei of different mammalian cells.

(c) It is known that infectious centers are reduced by a factor of 3 to 5 logs upon extraction of RNA-viruses with phenol. In the case of tobacco mosaic virus, however, efficiency of infection was increased thousand fold by combining V-RNA with its protein partner[17]. Our results seem to indicate that two main factors are involved in the efficiency of infection with V-RNA: protection from extracellular nucleases and absorption on the host as well. The increased uptake of protein-bound nucleic acids that we have observed is in agreement with recent reports of similar enhancements observed for DNA, R-RNA and V-RNA combined with gelatin[18], protamin[19] and calfthymus histones[20].

(d) The system we have studied could be used as a model for investigating the action of interferon(s), which are described as protein(s) of low molecular weight synthesized by cells treated with inactivated viruses and able to inhibit multiplication of other alive challenge viral species. One hypothesis that we had postulated is that interferon(s), like our methylated albumin, may merely undergo combination with nucleic acids in a complex stable to intracellular enzymes. Further work is necessary to clarify this point.

Résumé. Les acides nucléiques se combinent avec l'albumine méthylée et forment des complexes qui ne sont pas hydrolysés par les nucléases, mais dissociés par des ions inorganiques. Ces nucléoprotéines pénètrent dans les cellules en quantité beaucoup plus élevée que les acides nucléiques correspondants, et contrairement à ceux-ci, elles ne sont pas catabolisées. La combinaison avec l'albumine méthylée rend inactif l'acide ribonucléique viral: les ions inorganiques, et non les cellules vivantes, sont capables de dissocier ce complexe et de libérer l'ARN infectieux.

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Influence of Chlorpromazine on Nuclear and Cytoplasmic Uptake of ^{35}S-Methionine[1]

Introduction. The most evident effects of chlorpromazine on cell metabolism seem to be an increase in the ATP content of certain tissues and a derangement of the electron transfer process[2–5]. In several instances morphological degenerative changes have been observed[6,7].

The results of the influence of chlorpromazine on protein synthesis are in conflict. MITINA[8] after studying several groups of rats injected with increasing doses of chlorpromazine, up to 20 mg/kg of body weight per day for 13 days, found no difference in the ^{35}S-methionine uptake by proteins in the experimental animals, as compared with controls. LINDAN et al.[9] observed *in vitro* an inhibition of the rate of glycine-1-^{14}C incorporation into rat brain cortex proteins, in a concentration of the drug that has little or no effect on the respiration of the brain or on the rate of breakdown of glycine-1-^{14}C into $^{14}CO_2$. ZÖLLER et al.[10] found a decrease in the incorporation of glycine-^{14}C and lysine-E-^{14}C following the injection of only 5 mg/kg body weight of chlorpromazine into rats.

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