Table III. The influence of PCMB and FMB on the ATP induced contraction of tailed particles

Specimen (proportion of tailed particles)	PCMB $3 \cdot 10^{-4} M$	FMB $1 \cdot 10^{-3} M$
Control	88.0 ± 0.9%	85.0 ± 5.0%
+ ATP	49.2 ± 8.7%	65.2 ± 3.0%
+ (ATP + inhibitor)	82.5 ± 7.6%	93.4 ± 1.7%

only PCMB, FMB, and NEM in the concentrations $3 \cdot 10^{-4} M$, $1 \cdot 10^{-3} M$ and $1 \cdot 10^{-3} M$ respectively, exhibited a marked influence on the interaction of the virus with ATP (Table III). In those experiments where inhibitors + ATP or the inhibitor itself have been added to a plasma with a virus population containing a high proportion of spherical particles, a complete transition of these particles into the tailed form has been observed (Figure 3). In accordance with the interpretation of the influence of Hg-compounds on SH-enzymes, it has proved possible to suppress the action of PCMB and FMB by $1 \cdot 10^{-3} M$ cysteine contemporary added with the inhibitor.

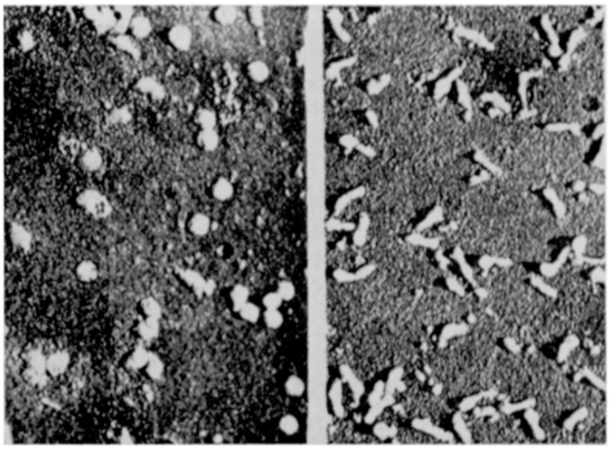

Fig. 3. The transition of spherical forms of virus particles into tailed forms, induced by the addition of PCMB to the plasma from which the virus particles were sedimented. Magnification ×30000.

All the inhibitors, i.e. $1.4 \cdot 10^{-3} M$ chloropromazine, $2 \cdot 10^{-3} M$ MJA, $7 \cdot 10^{-3} M$ atebrine, and PCMB, FMB and NEM in concentrations as mentioned above, decreased the virus ATP-ase activity by 50–60%; but only PCMB, FMB and NEM suppressed the ATP-virus interaction resulting in structural changes. This fact may indicate that the virus-bound ATP-ase activity, or its main proportion inhibited by the above-mentioned inhibitors, does not participate directly in the mechanism of the structural changes described in this paper.

Zusammenfassung. Sphäroidale und phag-ähnliche Form des Hühner-Leukosis-Virus (BAI, Stamm A) wird der Existenz eines kontrahierbaren Systems (Interaktion mit ATP) zugeschrieben. Formveränderungen des Viruspartikels unter ATP-Einfluss werden in vitro dargestellt, wobei Parachlormercuribenzoat, Phenylmercuriborat und N-Äthylmaleimid als Inhibitoren wirken.

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Complex Formation Between Glycerol and Metal Ions as Studied by Means of ESR, NMR, and Optical Absorption Spectroscopy

Recently we have shown that glycerol modifies the radiation response of catalase[1] as well as of lactate dehydrogenase[2]. The formation of a complex between glycerol and the metal ions, present in the enzymes and responsible for their biological activity, seems to be the cause for the protective effect. In this communication further evidence for the existence of a metal-glycerol complex will be given. Metal-glycerol solutions ($CuCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $FeCl_3 \cdot 6H_2O$ and $MnCl_2 \cdot 4H_2O$) were investigated by means of electron spin resonance (ESR), nuclear magnetic resonance (NMR), and optical absorption spectroscopy studies.

A Cary 14 spectrophotometer was used for the optical absorption studies of Fe^{3+} and Cu^{2+} in water and glycerol, respectively. The absorption spectrum of glycerol was also recorded in order to avoid any interference of glycerol with the other spectra. A small amount of HCl was added to the water solutions to prevent hydrolysis. The ESR spectra of Cu^{2+}, Fe^{3+}, and Mn^{2+} in water and glycerol, respectively, were determined with a Varian V 4500 100 kc ESR spectrometer. The position of the signals was determined with a standard DPPH reference signal for which $g = 2.0036$. The NMR measurements were carried out on a Varian V 4250 low-resolution NMR spectrometer. The wide-line spectra have been calibrated against external tetramethylsilane.

An absorption peak for Fe^{3+}-water solution (curve C) is present at about 3352 Å (Figure 1). The absorption peak of an Fe^{3+}-glycerol solution (curve D), however, was shifted to a longer wavelength (red shift) as compared to the Fe^{3+}-water solution. The difference between the two absorption peaks is about 167 Å. The absorption peak for glycerol (curve A) had no peak in the wavelength range under investigation. The wavelength shift and the occurrence of a color in the iron-glycerol solutions (light yellow) while iron-water solutions were colorless demonstrate that

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[1] W. Lohmann, A. J. Moss jr., W. H. Perkins, and C. F. Fowler, Biophysik 2, 16 (1964).

[2] W. Lohmann, A. J. Moss jr., and W. H. Perkins, J. nuclear Med. 5, 304 (1964).

a complex between the metal-ions and glycerol was formed. It should be pointed out that the absorption by this complex is changed with time as can be seen in Figure 1, curve E. This curve was registered about one week after measuring curve D. The absorption maximum at 3519 Å is still visible, its intensity, however, was considerably reduced. No explanation can be given yet for this observation. Similar results were obtained for Cu^{2+}. The absorption peak is at 2550 Å for a copper-glycerol solution (curve C) while neither a copper-water solution (curve B) nor glycerol (curve A) have a peak at this wavelength.

The formation of a complex between metal ions and glycerol could also be demonstrated with ESR and NMR measurements (Figures 2 and 3). The well-known Mn^{2+} (-water) spectrum with its 6 peaks is shown in the upper right part. However, Mn^{2+}-glycerol solutions gave a completely different ESR pattern; a single, very broad signal was obtained. The normal Mn^{2+} 6 line hf spectrum was observed to be superimposed on this broad signal at 77°K. Glycerol itself did not give a signal since it is not paramagnetic. The hf pattern of Cu^{2+} visible in glycerol has completely disappeared in water. The iron signal at a higher g-value is expressed only in glycerol.

The collapse of the hyperfine pattern of all three metal ions investigated was probably induced by a rapid relaxation of the ligands brought about by exchange processes. The Cu^{2+} and Fe^{3+}-glycerol solutions exhibited an hf pattern indicating a stable complex, while the Mn^{2+}-glycerol solution showed only one line indicating only a weak interaction. The variation in the line-width – and the 'red shift' in the optical absorption spectra – pointed out a change in the crystal-field energy levels by spin-orbit mechanisms[3].

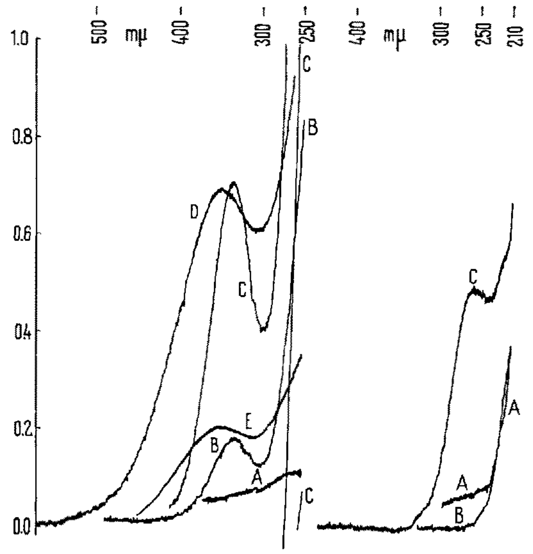

Fig. 1. Optical absorption spectra of different metal ions in glycerol and water, respectively. Right figure: A, Glycerol; B, $CuCl_2$ in H_2O (0.096 mg/ml); C, $CuCl_2$ in Gly. (0.096 mg/ml). Left figure: A, Glycerol; B, $FeCl_3$ in H_2O (0.128 mg/ml); C, $FeCl_3$ in H_2O (0.365 mg/ml); D, $FeCl_3$ in Gly. (0.128 mg/ml); E, same as D, 1 week old.

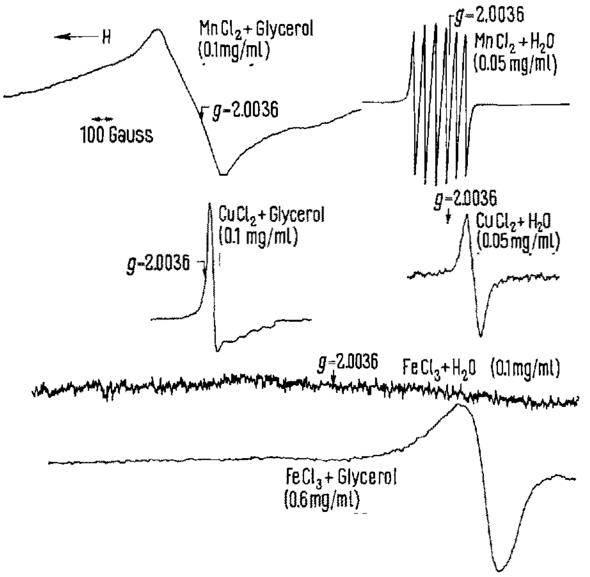

Fig. 2. Electron spin resonance signals of Cu^{2+}, Mn^{2+}, and Fe^{3+} in water and glycerol, respectively. The curves represent the second derivative of the actual resonance curves.

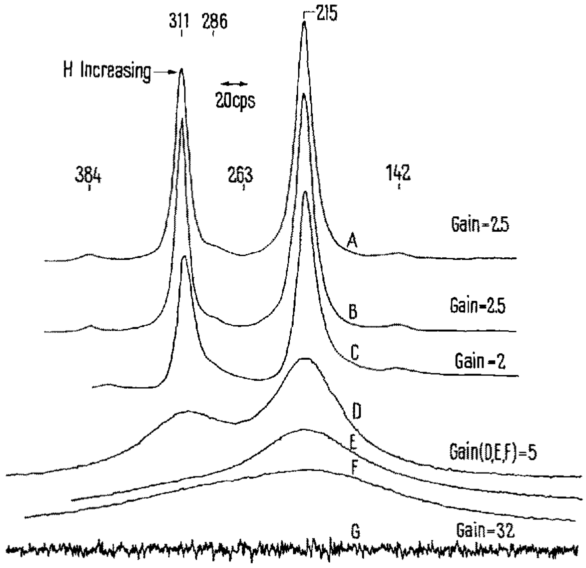

Fig. 3. Nuclear magnetic resonance spectra of different metal ions in glycerol. A, Glycerol; B, $FeCl_3$ in Gly. (2 mg/100 ml); C, $CuSO_4$ in Gly. (2 mg/100 ml); D $MnCl_2$ in Gly. (4 mg/100 ml); E, $CuSO_4$ in Gly. (4 mg/ml); F, $FeCl_3$ in Gly. (4 mg/ml); G, $MnCl_2$ in Gly. (4 mg/ml).

Zusammenfassung. Es wurden die ESR-, NMR- und die optischen Absorptionsspektren für Fe^{3+}, Cu^{2+} und Mn^{2+} in Wasser- oder Glycerollösungen bestimmt. Die untersuchten Metallionen bilden einen Komplex mit Glycerol.

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April 6, 1964.

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