after vagotomy and adrenalectomy[2] and reserpinization[1], it would seem possible that persistent acceleration is a direct effect of heating.

The added vitamin B_6 compounds may interact with or block the action of endogenous vasoactive amines liberated during the hyperthermia stress of the microwave irradiation. Support for this possibility is found in reports that adrenalectomy eliminates the increase in cardiac output due to microwave irradiation whereas ganglionic blockade[2] or reserpinization[1] merely reduces the increase in output; and that the catecholamines and histamine content of the plasma increase[7] during induced hyperthermia of a similar degree.

It is usually conceded that the administration of vitamins in excess of the amount provided in the usual daily minimal requirement is of little consequence. The absence of any demonstrable effect from the administration of the vitamin at normal body temperature reinforces this view. However, the inhibition of the increase in cardiac output during a rapidly induced change in status emphasized other possible functions of these biologically active compounds. These studies do not indicate whether the effect obtained during hyperthermia is beneficial. The duration of the effect has not yet been delineated[8].

Résumé. L'administration intraveineuse de pyridoxine et pyridoxal (10 mg/kg) à des rats anesthésiés inhibe l'augmentation du rendement du coeur. En même temps, une hyperthermie est provoquée par l'irradiation du corps entier avec des ondes de longueur minime (2,450 Mc. c.w. 0.08/w/cm²). Cet effet peut être attribué à l'interaction de ces composés d'amines vasoactifs libérés pendant l'irradiation.

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Changes Effected in vitro upon some Morphological Properties of Particles of Virus BAI-Strain A

The work of BEARD et al. has supplied us with basic facts about the structure and properties of virus BAI-strain A particles[1–6]. Our previous papers dealing with the pleiomorphism of these particles[7–8] have pointed out the relation between the functional state of leukemic cells, the stage of the leukemic process, and the occurrence of spherical or tailed forms of virus particles isolated from blood plasma. This paper deals with experiments directed towards influencing the form of these virus particles in vitro.

Materials and methods. White Leghorn chickens 17–20 days old, chosen for their susceptibility to virus BAI-strain A (which causes myeloblastosis when applied intravenously[6,9,10]), were used in our experiments.

The primary material used for the preparation of the individual plasmas was the cytoquantitatively defined blood taken from experimental animals[11]. The heparinized blood was sedimented at 1500 *g* for 30 min and at 2000 *g* for 30 min and the supernatant was used further.

Experiments directed towards influencing the form of virus particles were carried out on two or more aliquot amounts of the same plasma. This method was chosen in order to preserve the virus in its natural medium, and to eliminate in this manner any influence that a purification in other media could have effected. To the first amount of plasma (used for reference) was added the solution of tris-(hydroxymethyl)-aminoethane-KCl-$MgSO_4$ in an amount necessary to obtain a final concentration of 1 m*M* of all decisive components. The same solution further containing the substrate (e.g. adenosine-triphosphate-Na) or the inhibitor was added to the experimental portion of the plasma itself, to obtain the final concentration of 1 m*M* as well. Incubation time in all experiments was 7 min at 37°C.

The virus was sediménted from the plasma at 34000 *g*. The sediment of virus particles thus obtained was immediately resuspended in a 0.15*M* solution of ammonium carbonate and ammonium acetate of pH 7.0. The specimens for the electron microscope were prepared by spraying this solution onto a collodion membrane with a high pressure spray-gun[12]. The fixation in OsO_4 vapours was followed by Au-Pd alloy shadow-casting.

The following compounds were used in our experiments:

ATP – adenosine-5'-triphosphoric acid – Sigma Co.
ADP – adenosine-5'-diphosphoric acid – Boehringer
AMP – adenosine-5'-monophosphoric acid – Fluka AG.
Tris – tris-(hydroxymethyl)-aminoethane
PCMB – *p*-chloro-mercuri benzoate Na
PMB – phenyl-mercuri borate
NEM – N-ethylmaleimid
MJA – iodo-acetic acid
Atebrine – chinacrinum hydrochloricum
Chloropromazine (Largactil) – Specia, Paris.

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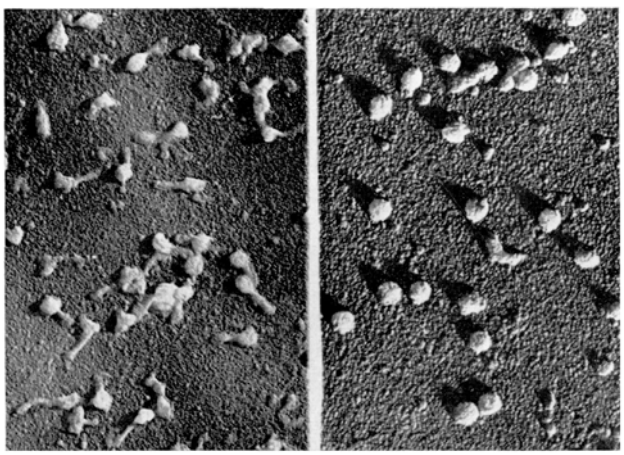
PCMB was added in a dioxane solution, since it has been proved that the admixture of the same amount of pure dioxane does not influence the shape of the particles under study.

Results and discussion. The addition of ATP + Mg^{++} to the BAI strain A virus containing plasma results in a change of the shape of the particles, i.e. the 'tail' of the phage-like particles originally present in the medium shrinks to 10–20% of the original length (Table I, Figure 1). Variations in any other characteristic dimensions of the virus have not been observed. Most expressed changes could be detected in virus populations containing the highest proportions of tailed particles (Figure 2), where the greatest number of tailed particles contracts after the admixture of ATP.

We have succeeded in inducing the above-mentioned changes in the shape of virus particles under conditions in vitro by the addition of ATP + Mg^{++} only. Changes provoked by ADP + Mg^{++} or AMP + Mg^{++} under the same conditions resulted in changes lying on the limits of statistical significance (Table II).

The degree of concentration of the 'tail' of phage-like particles, observed in our experiments, is in agreement with the data on the contraction of living cells[13]. As far as is known, the addition of ATP (or ITP) does not result in any morphological changes of other systems than the contractile ones[13]. According to these facts, our results led us to consider the existence of an enzymatically governed interaction of ATP with some contractile system present in the virus particle.

The contractile systems are always accompanied by an adenosinetriphosphatase (ATP-ase) activity, the role of which in the process of contraction and relaxation, however, is still disputable[14,15]. The assumption that such a system does exist in our experiments is supported by the fact that a high membrane bound ATP-ase activity[16,17], having some of the properties of the actomyosine ATP-ase[16], always accompanied the BAI strain A virus. Our attention was directed, therefore, towards the influence of ATP on the shape of the virus particle under the same conditions, but in the presence of some enzyme inhibitors. Some strong ATP-ase inhibitors known from literature are: PCMB, FMB[18,19], atebrine[20,21], chloropromazine[21,22], NEM[23,24], and MJA[18]. From the inhibitors examined



a b

Fig. 1. The same virus population (a) before and (b) after the addition of ATP to the plasma from which the virus particles were sedimented. Magnification ×30000.

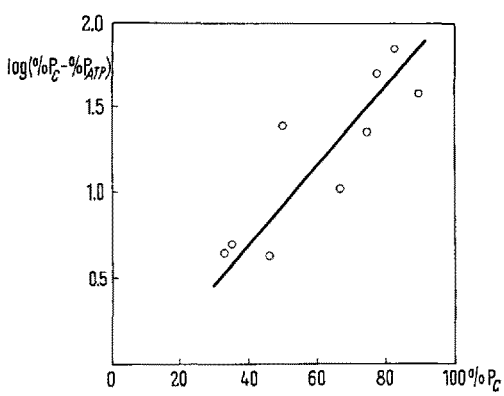


Fig. 2. The decrease in the proportion of tailed particles ($\%P_{ATP}$) induced by ATP with relation to their original quantity in the plasma ($\%P_C$).

Table I. Changes in the shape of BAI-strain A virus particles induced by ATP + Mg^{++}

	Spherical particles (diameter)	Tailed particles: diameter of the 'head'	Tailed particles: length of the 'tail'
Control	1240 ± 40 A [a]	1240 ± 40 A	1600 ± 40 A
+ ATP	1270 ± 60 A	1190 ± 60 A	400 ± 60 A

[a] Average $X \pm S_M$ (calculated per 500–1000 particles)

Table II. The comparison of the influence of ATP, ADP and AMP on the relative proportion of tailed particles in the virus population

Substrate	% of tailed particles before the addition of the substrate	% of tailed particles after the addition of the substrate	Difference %
ATP	89.5 ± 0.1	59.1 ± 2.0	36.0
ADP	93.5 ± 0.1	86.5 ± 3.5	7.5
AMP	93.5 ± 0.1	84.8 ± 0.3	9.4

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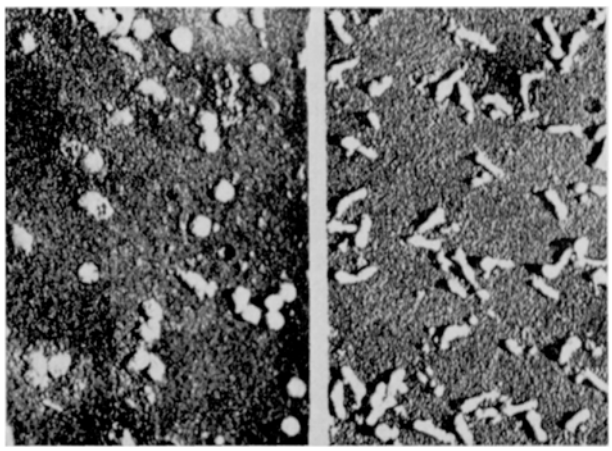
Table III. The influence of PCMB and FMB on the ATP induced contraction of tailed particles

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%



Native + PCMB

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.

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.

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 ($CuCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $FeCl_2 \cdot 6H_2O$ 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 96% 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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