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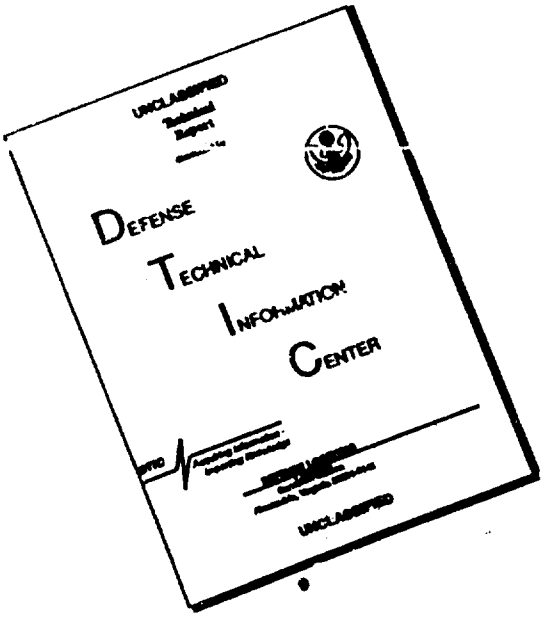

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Complexes of influenza virus with organic bases and cationic detergents.

Biokhimiya 13, 477-481 (1948)

It has been reported by Licitzin and Alexandrov (1) that protamines easily enter into compounds with high molecular weight proteins. These complexes precipitate out of solution in the form of salt-like complexes insoluble in water, but soluble in concentrated solutions of salts and in alkalies.

In the work of our laboratory (2) similar facts were established for influenza virus. Furthermore, in virus, as in biologically active protein there were disclosed additional new facts, which could not be established during work with biologically inactive proteins.

We bear in mind the reversible inactivability of the virus, caused by the stable blocking of the carboxyl groups of the virus by the formation of acidic basic protein salts.

It has been reported by Petrunkin and Petrunkin (3) that such organic bases as atropine, quinine and strychnine, or such bases of the organism, as guanidine and adrenalin, enter into salt-like compounds with gelatin in the alkaline zone of the isoelectric point of the protein. The significance of the reaction of the medium in the finding of alkaloid with proteins has been extraordinarily graphically demonstrated in the experiments of Labes (4). Afterwards Licitzin (1) reported that such organic bases as Piper~~r~~idine, nicotine, cocaine, quinine and others are able to form salts with high molecular weight proteins.

Unlike protamine complexes these salts of proteins in water with some organic bases are readily soluble, but with others (quinine, cinchonine, morphine) -- are difficultly soluble.

Leont'ev (5) has reported, that aqueous solutions of complex compounds of nicotine with casein appeared harmless by the hypodermic introduction both for warmblooded and coldblooded animals. In this connection it is interesting to investigate also the conduct of the virus proteins in the process of salt-formation with organic bases, that is with compounds, much more simple than

with protamines or other basic proteins. The task in addition was, in the first place, to investigate the possibility of the formation of analogous complex compounds with organic bases by viruses, and, secondly, to determine their chemical and biological properties. For these investigations we selected the following series of basic compounds: arginine, piperidine, anabasine, caffeine and quinoline.

By way of a source of virus there was used a suspension of influenza virus out of mouse lungs (1:100), inoculated with strain PR8. To a known volume containing virus suspension was added 0.1% solution of one or another base in ratio 1:1, and after mixing, the mixture was placed in a refrigerator overnight. On the next day it was centrifuged and examined for precipitates. Examinations of the precipitates for virus activity were carried out according to Hirst and with the biological method on mice but on the supernatants only titrations according to Hirst were carried out. In contrast with the virus protein complexes it was evident that there are sharply different degrees of precipitability of the virus from solution (as the organic base complex), similarly, with its biological activity. Concerning this, data are cited in tables 1 and 2.

As we see from the cited data, the ability to precipitate virus increases in proportion to the complexity of the structure of the base. As for the stability of the formed complexes, they appeared easily cleavable in vivo owing to easy dissociation of these compounds through the weak connection at the tertiary.

With this configuration, in distinction from alkaline proteins, organic bases are not able firmly to block the carboxyl groups of virus proteins and cannot consequently of this adduce to the formation of biologically inactive complexes. For this there is required a structurally more complex molecule, which in our preceding works were proteins of basic type: globin, papain, cytochrome and clupein.

During recent years in the literature there have been accumulated voluminous material on the question of the effect of detergents (inverted soaps) on bacteria, viruses, and protein material. All detergents appear to be

Table 1

Precipitability of virus of influenza with organic bases and their biological activity (titrated in mice).

Dilution 10^{-3}	0.5	2	12	62	312	1662	7612
Original virus	332	330	333	210	310	322	221
Piperidin complex	100	100	#	320	#	#	#
Anabasin complex	333	310	300	322	320	#	#
Caffein complex	321	333	333	220	221	332	331

Note: Ciphers (i.e., numerals) denote loss of mice from influenza infection and the amount (number) of affected (diseased) lobes of the lungs;

0 = mouse lost not from influenza infection;

= mouse remaining alive.

Table 2

Titer of supernatant fluid by Hirst Technique

Dilution	1/20	1/40	1/80	1/160	1/320	1/640	1/1280
Original virus	+++	+++	+++	+++	+++	+++	-
Piperidin complex	+++	+++	+++	+++	++	+	-
Anabasin	+++	+++	+++	+++	-	-	-
Caffein complex	+++	++	-	-	-	-	-

powerful bactericidal agents, the extent of bactericidal-power of which varies in relation to the length of the chain of the fatty acids. Detergents have a denaturing effect on proteins causing such alterations in the protein molecule as opening of SH groups. In relation to this, whether we are dealing with anionic or cationic detergents, a ppt precipitation of proteins takes place with the cationic or the anionic form and in appropriate pH ranges about the isoelectric point of the protein.

The effect of soaps and detergents on viruses was studied by a series of authors. All of these works talk about large or small virucidal effects of detergents on viruses in vitro, whereupon ordinary soaps have greater activity effects than the inverted (6). It was reported (6) that virucidal activity of detergents is associated with the length of the paraffin chain (the inactivating effect begins only with C_{12} and higher) and, consequently, with the complexity of structure of the detergent. Cationic detergents possess much greater virucidal power, than the anionic (7). The relation of different viruses to detergents is sharply differentiated: one virus is inactivated already with weak concentrations of detergents, another may be completely susceptible to them (8). Phages appeared generally resistant to detergents (7). All of these results pertain to the effect of detergents on viruses in vitro. As to the effects of detergents on viruses in vivo, all of the experiments, conducted by various researchers on different viruses and with different detergents have been reported with negative results.

We have studied the effect of anionic and cationic detergents on influenza virus both in vitro and in vivo. The collection of detergents was kindly given to us by Prof. A. G. Pacwinkii, for which we express to him our thanks.

Suspensions of influenza virus (from 1:20 to 1:100) were prepared with different amounts (from 0.4 to 1.0 ml) of 1% detergent solution. We recorded the settling out of precipitation, the titer of the virus according to Hirst and biological activity on mice. Researchers have reported, that virus is precipitated by detergents only in the anionic form. With anionic detergents, virus is not at all precipitated.

Cationic detergents precipitate likewise nucleoproteins and proteins from normal suspensions of lungs, but precipitates of these do not give the

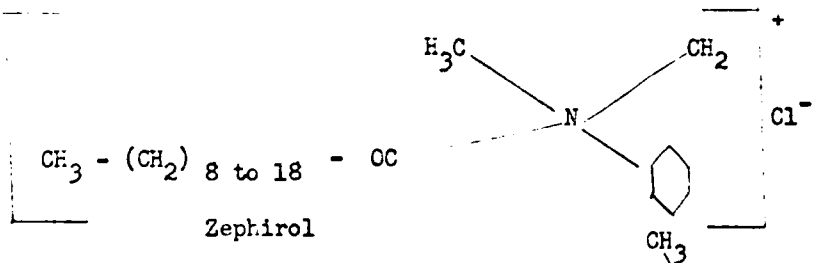
Hirst reaction, characteristic of influenza virus. The precipitates from virus nucleoproteins, produced through the effect of cationic detergents, react with Hirst reagent in the same titer (providing there is completeness of precipitation) as the original virus by itself. Certain pertinent data here are cited in table 3.

In this experiment (table 3) the titer of the untreated virus suspension was equal to 1:800. As we see, only above a dose of 0.8 ml of solution of Zephirol was complete precipitation of virus obtained.

As to the biological activity of the influenza virus, treated with cationic (Zephirol) and anionic (Igepon) detergent, in the first instance, that is when we have blocking of the carboxyl groups by the complex cation of Zephirol, biological activity is suppressed in marked degree; in the second case, when there is no binding of the carboxyl groups, it remains unchanged.

In table 4 are cited data of a pertinent biological experiment in which a suspension of influenza virus was treated with 1% solution of Zephirol up to complete precipitation of the virus. The precipitation of the complex in the first instance was suspended in water, and in the other in a solution of phosphate buffer pH 7.5. With these suspensions there was performed a biological titration in mice.

From table 4 it is seen that virus-detergent complexes possess properties, analogous to the virus-protein complex, that is that they are insoluble or slightly soluble in water and easily split in salt solutions. Nevertheless, these complexes are not very stable inasmuch as during introduction <u>in vivo</u> there was observed partial reactivation of the virus.



Zephirol

The anionic detergent Igepon T $[C_{17}H_{33}CO - N - C_2H_4SO_3^-]Na^+$ did not appear to affect biological activity of the virus. A suspension

Table 3

Precipitation of Influenza Virus by Different Detergents

Titer of original virus 1:640

Group	Name of detergent	Ppt.	Hirst titer in the ppt.	Hirst titer in supernatant fl.
Cationic	Zephirol	+	1:640	0
	Ceramin	+	1:320	1:160
	Cetavlon	+	1:160	1:320
Anionic	Igepon T	-	0	1:640
	Igepal	-	0	1:640
	Neopol	-	0	1:640
	Gardinol	-	0	1:640

Degree of pptn. of virus in relation to quantity of given detergent is observable from the following data:

Quantity: ml 1% solution of Zephirol on 5 ml virus suspension 1:1000

	0.4	0.6	0.8	1.0
Titer of the ppt. by Hirst	1:200	1:400	1:800	1:800

Table 4

Biological Activity of Virus-Zephirol Complex
(labelling the same as that in table 1).

Dilution 10^{-3} x	1	3	4	5	6	7
Untreated virus	444	444	433	432	310	#
Virus-Zephirol Complex in Water	444	431	10#	#	#	#
Virus-Zephirol Complex in Buffer	442	432	433	333	32#	#

of influenza virus 1:80, prepared with 1% solution of Igepon T in ratio 2:1, showed biological activity. Such suspensions were inoculated intranasally into mice, which perished from influenza infection with characteristic changes in the lungs in 5 to 7 days; the lungs of these mice were titrated according to Hirst in dilution 1:1600 with the control titer of untreated virus 1:640.

In the present instance we could state not only complete freedom from inhibition of the reaction, but even some activation of virus in passage, as usual. An analogous reaction of detergents on the virus of tobacco mosaic was established by Pfankuch and Kausche (9). The virus precipitated from solution with just technical concentrations of detergent, as that usual for proteins (0.1 to 0.01%). Such a complex easily decomposed in phosphate buffer at 70 to 80%.

In such a manner, in agreement withour data of Pfankuch and Kausche, viruses react with detergents as with simple proteins, with the distinction that they are less easily denatured. Researches on the effect of detergents on virus are of interest in that they point out the opportunity to combine and to block viruses not only with basic proteins, but by compounds of non-protein type, possessing structural complexity and complex cations, with ability to accomplish in these complexes the role of basic proteins in their cationic form.

It is hardly necessary to speak about the application of detergents for the prevention of virus infections. However, theoretically, it is conceivable that such compounds could be synthesized which would be harmless for cells in general but which with a structure analogous to the detergents could form molecular complexes with the carboxyl groups of viruses. This would be in line with the chemotherapeutic objectives of alkaloid (quinine) and organic bases (antipyrine, urotropine, etc) acting for the most part through tertiary nitrogen and not necessarily beingable to completely block the carboxyl groups of the virus.

CONCLUSIONS

The virus of influenza precipitates from solutions completely only with cationic detergents.

Viruses, like higher proteins, form complex compounds with organic bases and cationic detergents. Stability of linkage of carboxyl groups of

the virus micelle with organic bases and cationic detergents is not great, and these complexes more or less easily split in physiological solutions of salts.

The search for antiviral chemotherapeutic agents must be directed along the road of search for compounds harmless for organisms, possessing cations that are very complex in structure, of high molecular weight, capable stably to combine with the carboxyl groups of the virus.