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ARBOVIRUS HAEMAGGLUTININ: INACTIVATION BY HIGH PRESSURES

C. HANNOUN, N. POUEY

Arbovirus Laboratory, Pasteur Institute, Paris; and Laboratory of High Pressures, C.N.R.S., Bellevue, France

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Summary. — The effect of high pressures (2000 to 7000 bars) on the haemagglutinins of 17 arboviruses belonging to different groups was studied by experimental exposure for 5 minutes in a steel cylinder. The viruses belonging to the same group have comparable susceptibilities but the differences are large between groups: in the more susceptible group (group B), the haemagglutinin is always completely destroyed at 2100 bars although in the more resistant (Bunyamwera group), the limit is between 4200 and 5250 bars. Treatment of Sindbis virus by Tween-ether does not cause any definite change in susceptibility. Treatment of Ťahyňa haemagglutinin by trypsin induces an increase of resistance to high pressures. The susceptibility of haemagglutinins to high pressures cannot be related to the size of the virion and is probably due to alterations in secondary or tertiary structure of protein units.

Introduction

Data concerning the physical and chemical structure of arbovirus virions are still incomplete and the 250 viruses of that family, subdivided in several groups, cannot yet be classified properly. It is therefore necessary to collect all the informations which can help to appreciate a) the homogeneity of the family of arboviruses, and b) the possible relationship between this family and other viral groups. It is possible to obtain indirect information on the structure of virions by the study of the susceptibility of the biological properties of the viruses to various chemical and physical agents. The high pressures are one of these agents, they are easy to control and to measure with accuracy: the available equipment can be used to produce high pressures from 1000 to 10,000 bars. We have studied the effect of high pressures of increasing values on preparation of arbovirus haemagglutinating antigens from several groups to determine in each case the critical pressures and to compare them to each other as well as to the values obtained for other viruses.

Materials and Methods

Haemagglutin preparations were obtained from infected suckling mouse brain suspensions by one of the two classical methods: sucrose-acetone treatment according to Clarke and Casals (1958) or fluorocarbon extraction according to Porterfield and Rowe (1960). The virus strains used are given in the caption to Fig. 1.

The Tween-ether treatment was performed according to Mussgay and Rott (1964) and the trypsin digestion was carried out for two hours at 37° C at an enzyme concentration of 512 μg/ml (Hannoun, 1968).

The pressure vessel was designed by the "Laboratoire des Hautes Pressions du C.N.R.S." (for a detailed description and schematic picture, see Pouey and Trimoreau, 1967). It is a steel cylinder which was autofretted to a pressure of 13,000 bars. The steel cap is a self sealing variety with a teflon gasket and a copper ring to prevent the packing leakage so that the higher the pressure the tighter the sealing. The pressure was generated by an oil intensifier and the samples were hydrostatically compressed.

Pressure treatment. The samples of haemagglutinin were placed in small bags made of from plaques of fine rubber gum (obtained through the courtesy of M. Warnery, Institut Français du Caoutchouc). The bags were then sealed by forceps pressure and, after checking their watertightness, immersed into the pressure medium made of 2/3 glycerol and 1/3 water. In the case of arboviruses, special attention was brought to the possible inactivating effect of the gum, and for some preparations (Ťahyňa for example) the haemagglutinin was isolated from the gum by a sheet of Parafilm; under these conditions no inactivation occurred, even with the most labile viruses. To prevent thermal inactivation, the bags were continuously maintained in an ice bath before and after high pressure treatment. During pressure treatment, no noticeable temperature increase was noted in the chamber. The control preparations were treated exactly like the experimental material (insertion into bags, ice bath) before titration. The pressure treatment was applied in all cases for a duration of 5 minutes.

The results were evaluated from the haemagglutinating titre of each of the antigens at the appropriate pH. The results were expressed as the reciprocal of the last haemagglutinating dilution: they were either equal to the control titre, or significantly lower (4-fold decrease) or completely negative. The *critical inactivation pressure*, according to our experimental system, is the pressure capable to decrease significantly the haemagglutinating titre of a preparation in 5 minutes.

Results

Action of high pressures on haemagglutinating antigens

Fig. 1 illustrates the overall results, which show clearly that the groups of arboviruses, as they were separated by Casals, are relatively homogeneous as far as susceptibility of their haemagglutinin to high pressure is concerned. The critical inactivation pressures can be summarized as follows:

Group A: from 2450 to 3150 bars;
group B: from 1750 to 2100 bars;
group Bunyamwera: between 4200 and 5250 bars;
Ťahyňa (Group California): around 2100 bars; and
group Sandfly fevers: from 3150 to 4200 bars.

Action of high pressures on modified haemagglutinins

The comparison of the effect of high pressures on Sindbis virus haemagglutinin before and after Tween-ether treatment showed only a very slight difference (+, ± and 0 as in Fig. 1):

Bars:	1750	2100	2450	3150	4200
Sindbis, untreated	+	+	+	0	0
Sindbis, Tween-ether treated	+	+	+	±	0

On the other hand, trypsin treatment of Ťahyňa virus induced a definite increase of resistance to the action of high pressure

Bars:	1750	2100	2450	3150	4200	5250	6300	7000
Ťahyňa, untreated	+	±	0	0	0	0	0	0
Ťahyňa, trypsin-treated	+	+	+	+	+	+	+	+

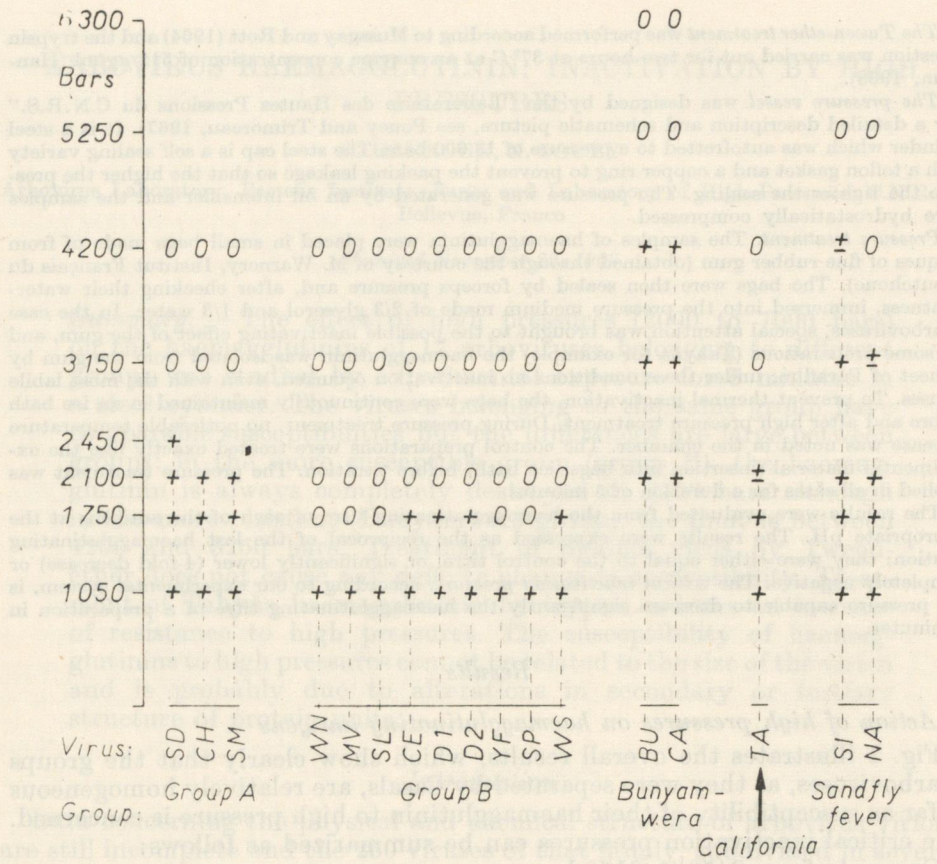

Fig. 1.

Action of high pressures on haemagglutinating antigens of arboviruses

\+ No change in haemagglutinin titre after pressure treatment

± Significant decrease in haemagglutinin titre after pressure treatment

0 Haemagglutinin no longer detectable at the lowest dilution (1 : 10)

Virus strains examined:

Group A: SD — Sindbis, CH — Chikungunya, SM — Semliki

Group B: WN — West Nile, MV — Murray Valley encephalitis, SL — St. Louis encephalitis, CE — Central European encephalitis, D1 — Dengue 1, D2 — Dengue 2, YF — neurotropic yellow fever, SP — Spondweni, WS — Wesselsbron

Group Bunyamwera: BU — Bunyamwera, CA — Čalovo

Group California: TA — Ťahyňa

Group Sandfly fevers: SI — Sicily, NA — Naples

Discussion

Several authors have studied the effect of high pressures on viruses (Basset and Maurin, 1958; Atanasiu *et al.*, 1951; Pouey and Trimoreau, 1967). They used inactivation criteria of different kinds (infectious titre, haemagglutinating potency, immunogenic activity) and the techniques were so

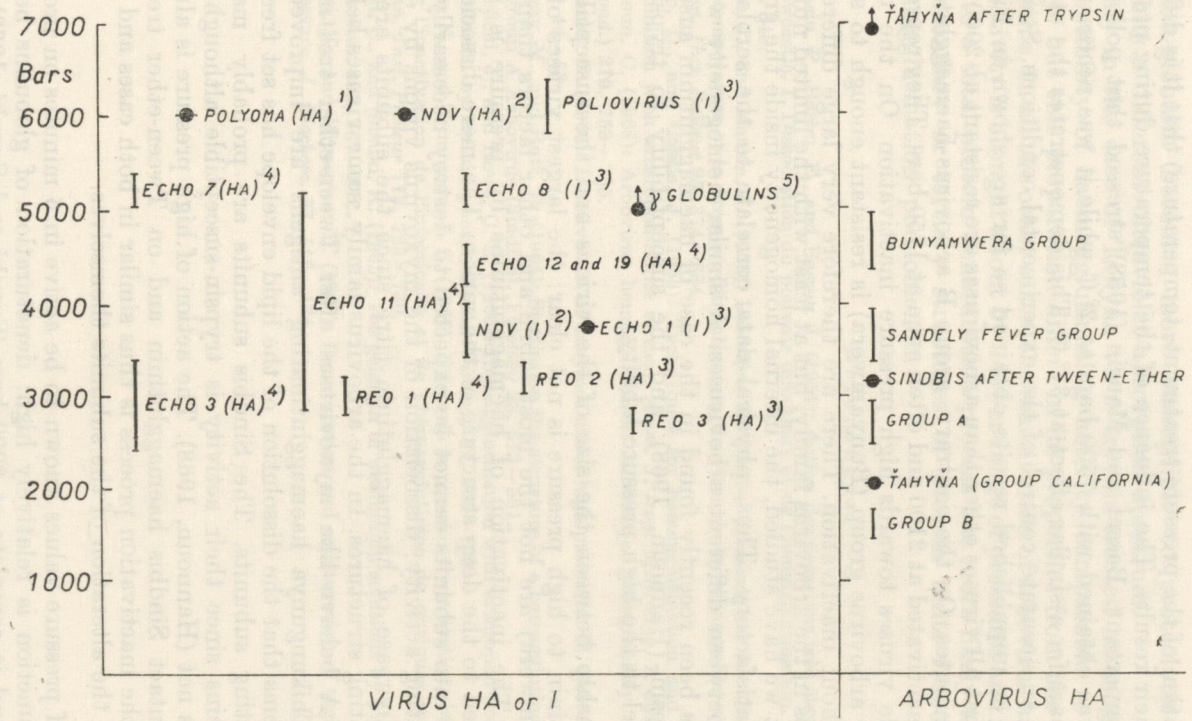

Fig. 2.
Action of high pressures on haemagglutinating activity (HA) or infectivity (I) of different viruses
1), 3) N. Pouey and J. C. Trimoreau, unpublished results
2) Atanasiu *et al.* (1951)
4) Pouey and Trimoreau (1967)
5) Suzuki and Miyosawa (1965)

different (duration of the pressure treatment, temperature) that it is difficult to compare their results. The influence of the temperature during pressing is especially important. Basset and Maurin (1958) stressed that poliovirus inactivation was obtained with 7500 bars at 2° C while it was necessary to reach 10,000 bars for a similar effect at 37° C. This demonstrates the utmost importance of an accurate control of the experimental conditions. Some of the published or unpublished results obtained so far are shown in Fig. 2: it is striking that all viruses other than arboviruses are resistant at 2000 bars for up to 30 minutes. On the contrary, group B arbovirus haemagglutinins are regularly inactivated at 2100 and often even at 1750 bars. They represent the most labile viruses towards high pressure inactivation. On the other hand, another arbovirus group (Bunyamwera) is resistant enough to stand 4200 bars without inactivation. There are therefore very large differences between groups in the arbovirus family, but at least with the limited number of viruses that we have studied, the internal homogeneity inside the groups is relatively satisfactory. Thus, physical data correlate to the serological relationships between different arboviruses. A similar homogeneity within the groups has been recently found in the case of haemagglutinin susceptibility to trypsin (Hannoun, 1968), and the susceptibility to trypsin is roughly parallel to the high pressure lability.

The relationship between the size of the virions and the susceptibility of haemagglutinin to high pressure is not clear: the largest viruses of the group (Bunyamwera) are not the most labile and other factors than size are involved. The inactivation of haemagglutinins by pressure is more probably related to the deep structure of the virion. The mere dissociation of the particle into subunits cannot be expected to destroy necessarily the haemagglutinating activity: dissociation of the myxovirus virions by ether promotes an increase of haemagglutinin titre since the subunits are the haemagglutinating structures. In the arbovirus family, some viruses belonging to group A behave like myxoviruses after Tween-ether treatment: Sindbis and Chikungunya haemagglutinating antigens are improved in titre and it seems that the dissolution of the lipid envelope has set free the haemagglutinating subunits. The Sindbis subunits are probably mainly made of proteins since their activity is trypsin-susceptible although the intact virion is not (Hannoun, 1968). The action of high pressure is almost identical on intact Sindbis haemagglutinin and on Tween-ether treated preparations: the inactivation process is thus similar in both cases and probably involves the alteration of the subunits themselves.

The range of pressure values known to be active in 5 minutes on protein structure or function is relatively high: denaturation of globulins begins at 5000 bars and is complete at 8000 bars (Suzuki and Suzuki, 1963) and various enzymes are inactivated only at 1000 bars (Kitamura, 1966). In these cases, the inactivation is not a one-step phenomenon since at first only some characteristics are altered or reversibly altered. These first changes are related to modifications of secondary or tertiary structure of the molecules: after high pressure treatment and reversible coagulation, redissolved

gamma-globulin becomes more susceptible to proteolytic enzymes and exhibits a different ultraviolet absorption spectrum.

Only in the case of Ťahyňa virus trypsin-treated haemagglutinin is the susceptibility of haemagglutinating activity comparable to protein sensitivity: but this preparation differs in its properties from the other haemagglutinins. In all the other cases, the inactivation of arbovirus haemagglutinating activity is brought about by a lower range of pressures and is therefore not due to a total protein denaturation process but more likely to an alteration of secondary or tertiary structures of protein units responsible for the haemagglutination phenomenon.

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