Bull. Org. mond. Santé
Bull. World Hlth Org. } 1952, **6**, 467-472

INHIBITORS OF INFLUENZA VIRUS HAEMAGGLUTINATION IN NORMAL ANIMAL SERA

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Manuscript received in September 1952

The antigenic analysis of strains of influenza virus was made easier by the discovery that influenza viruses agglutinate red blood-cells from different animals, and that this agglutination can be inhibited by immune sera.[8, 9, 12] This apparently simple method can, however, be upset by the existence of several non-specific inhibitors of agglutination in normal animal and human sera,[1, 2, 4, 6, 7, 10, 13, 15] neglect of which can distort the antigenic relationships between strains. As a preliminary to a comparison of different immune animal sera in elucidating antigenic relationships between strains, a study was made of the inhibitors of agglutination in normal animal sera, the results of which are given in this paper.

Materials and Methods

Virus

The following strains were used :

Influenza A : A/England/1/51 (E3); A/England/1/51 mouse-adapted (E3,M15,E2)

Influenza B : Crawley (England 1946)

The allantoic fluids used were from chick embryos inoculated at 10 days and incubated for a further 3 days at 35°C.

After harvesting, the virus was absorbed with 2% chicken red blood-cells and eluted into saline; the eluates were used throughout the tests, except for the study of Francis inhibitor (see page 468).

Red cells

Fowl cells were made up in 0.5% suspension in normal saline.

The concentration of cells was calibrated by a photo-electric densitometer. The cells used were from fowls previously known [3] to be sensitive to inhibitor and to vaccinia.

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Sera

The sera studied were from the following animals, bred at the farm of the National Institute for Medical Research, Mill Hill : rabbits, hamsters, guinea-pigs, mice, ferrets, and fowls.

In order to reduce individual variation as far as possible, several animals of the same species were used and after bleeding their sera were pooled. The following numbers of animals of each species were used :

Rabbits	3	Mice	30
Hamsters	9	Ferrets	4
Guinea-pigs	3	Fowls	3

The sera, after having been absorbed by 5% chicken red blood-cells to remove chicken cell-agglutinins, were kept at −70°C.

Vibrio cholerae *filtrates*

The filtrates were prepared by the technique described by Burnet & Stone.[5] Instead of using *Vibrio cholerae* filtrates[14] we used red-cell eluates,[11] prepared (in accordance with Burnet & Stone) in the following way : Washed, packed fowl cells were added to filtrates of *V. cholerae* at 2°C in a final concentration of 5%. After 30-60 seconds the cells were centrifuged at 2°C and the supernatants were removed. Saline was added to the same volume and elution was carried out for half an hour at 37°C. After this period the cells were centrifuged and the eluates titrated for ability to destroy non-specific inhibitor in normal ferret sera. This titration was carried out with all animal sera to find the best dilution of receptor-destroying enzyme (RDE) to employ in tests.

Titration of non-specific inhibitors

Serial twofold dilutions of serum (0.25 ml) were prepared in saline, and an equal volume of 0.5% fowl cells was added, followed immediately by a third equal volume of eight agglutinating doses (AD) of virus. Tests were carried out at room temperature, and the readings were made by the pattern method after standing for 60-70 minutes, the end-point being taken as 50% agglutination. The titres in tables I, II, and III are expressed as the reciprocal of the initial dilution of serum present at the end-point.

Titration of Francis inhibitor

The same technique as for the non-specific inhibitors was used, with the following variation : To the serum dilution, virus was added and was left to stand for half an hour; the chicken red blood-cells were added afterwards. The Crawley virus was heated beforehand for half an hour at 56°C in the form of infected allantoic fluid, since with eluates heating produced a sharp drop in agglutinin titre.

Experimental

The sera of all animals in this experiment were used without any treatment in haemagglutination-inhibition tests with different viruses; in order to reduce possible variation, tests were carried out at the same time. Viruses A/England/1/51 and A/England/1/51 M15 were used to show the difference in inhibitor content between unadapted and mouse-adapted strains,[6] and Crawley virus because heated influenza B viruses have been shown to be more sensitive to Francis inhibitor.[7] Table I shows the results obtained.

TABLE I. HAEMAGGLUTINATION-INHIBITION TITRES WITH NORMAL SERA FROM SEVERAL SPECIES OF ANIMALS AND DIFFERENT VIRUSES

Virus	Animal sera at 0° C					
	rabbit	guinea-pig	ferret	mouse	fowl	hamster
A/Eng/1/51	240	280	60	200	15	15
A/Eng/1/51 M15	60	30	15	<10	<10	<10
Crawley	240	120	30	60	15	<10

The sera showed titres which varied with the animal species and the virus used. The most striking finding was the difference in content of serum inhibitor between A/England/1/51 and the mouse-adapted lines of the same strain. The first to describe this difference was Chu,[6] and for this reason this inhibitor is referred to as " Chu inhibitor ". Chu inhibitor is present in rabbit, guinea-pig, mouse, and ferret sera. Fowl and hamster sera showed only a very small amount of this inhibitor.

Tests were next carried out to determine the presence of Francis inhibitor in the sera, using heated Crawley virus to indicate its effect.[7] The results obtained are given in table II.

TABLE II. HAEMAGGLUTINATION-INHIBITION TITRES WITH NORMAL SERA FROM SEVERAL SPECIES OF ANIMALS AND CRAWLEY VIRUS HEATED AND UNHEATED

Crawley virus	Animal sera					
	rabbit	guinea-pig	ferret	mouse	fowl	hamster
Unheated	240	120	30	60	15	<10
Heated	640	360	1280	70	640	<10

Ferret and fowl sera showed large amounts of Francis inhibitor; small amounts only were present in rabbit and guinea-pig sera, and it was not detectable in significant amounts in mouse and hamster sera.

Some Properties of Chu and Francis Inhibitors

Chu [6] has shown that the inhibitor for unadapted virus in mouse serum (Chu inhibitor) is heat labile and Anderson [1] has shown that Francis inhibitor can be inactivated by RDE. The effect of heat and RDE action on these sera was therefore investigated. The results are shown in table III.

TABLE III. HAEMAGGLUTINATION-INHIBITION TITRES WITH NORMAL SERA FROM SEVERAL SPECIES OF ANIMALS AND DIFFERENT VIRUSES, SHOWING THE EFFECT OF VARIOUS TREATMENTS

Animal	Treatments used					Virus
	heated for 30 minutes at					
	0°C	56°C	62°C	65°C	RDE*	
Rabbit	240	160	30	15	80	A/England/1/51
Ferret	60	30	15	15	<10	
Guinea-pig	280	50	30	25	30	
Mouse	200	100	50	50	<10	
Fowl	15	<10	<10	<10	<10	
Hamster	15	<10	<10	<10	<10	
Rabbit	60	30	<10	<10	<10	A/England/1/51 — mouse-adapted
Ferret	15	10	<10	<10	<10	
Guinea-pig	30	<10	<10	<10	<10	
Mouse	<10	<10	<10	<10	<10	
Fowl	<10	<10	<10	<10	<10	
Hamster	<10	<10	<10	<10	<10	
Rabbit	240	120	15	<10	<10	Crawley
Ferret	30	30	20	20	<10	
Guinea-pig	120	40	15	15	<10	
Mouse	60	20	<10	<10	<10	
Fowl	15	<10	<10	<10	<10	
Hamster	<10	<10	<10	<10	<10	
Rabbit	640	460	160	80	<10	heated Crawley
Ferret	1280	1280	1280	1280	<10	
Guinea-pig	360	360	360	360	<10	
Mouse	70	20	<10	<10	<10	
Fowl	640	640	640	640	<10	
Hamster	<10	<10	<10	<10	<10	

*** RDE treatment involves overnight incubation with RDE at 37°C, followed by heating for one hour at 56°C in order to inactivate the RDE.**

From this table it is seen that the two types of inhibitor show distinctive properties :

(*a*) The Chu inhibitor, e.g., that found in mouse serum, is much more active against unadapted than mouse-adapted strains of influenza A virus, is no more active against heated than unheated influenza B virus, and is reduced in titre by heating, although the susceptibility to heat differs in different sera. The action of RDE plus heat produces some effect, but it is difficult to dissociate the action of the enzyme from that of the heating. It is essential in these tests to heat in order to inactivate the RDE; citrate could not be used for this purpose since it has an action of its own (Mulder & van der Veen [14]).

(*b*) The Francis inhibitor, e.g., that found in fowl or ferret sera, is much more active against heated than unheated influenza B virus, is only slightly more active against unadapted than mouse-adapted strains of influenza A virus, is heat stable (65°C for 30 minutes), and is readily destroyed by RDE.

Some sera show behaviour which suggests that they contain both inhibitors. Although it is realized that knowledge of these inhibitors is far from complete, an attempt has been made to assess roughly the content of the two inhibitors in these sera; this is shown in table IV.

TABLE IV. ROUGH ESTIMATE OF THE CONTENT OF CHU AND FRANCIS INHIBITORS IN NORMAL SERA FROM SEVERAL SPECIES OF ANIMALS

Animal sera	Chu inhibitor	Francis inhibitor
Rabbit	++	+
Ferret	±	++++
Guinea-pig	+++	++
Mouse	+++	-
Fowl	±	+++
Hamster	±	-

Experiments with Crude V. cholerae Filtrate and Kaolin

Chu [6] has described the fact that crude filtrates of *V. cholerae* and kaolin reduced the inhibitory titre of normal mouse serum. In limited experiments with normal rabbit serum it was found that kaolin treatment reduced the inhibitory titre but was less effective than heat. Also, crude *V. cholerae* filtrate showed no significant action, although J. Mulder (personal communication) has found that it may eliminate completely the non-specific inhibitor in rabbit serum. These differences are at present under investigation.

SUMMARY

A study of the inhibitors of influenza virus haemagglutination in a number of animal sera has shown two types of inhibitor :

(*a*) Chu inhibitor, which is generally heat labile and is more active against unadapted than mouse-adapted influenza A viruses.

(*b*) Francis inhibitor, which is heat stable and is more active against heated than unheated influenza B viruses.

An attempt has been made to assess roughly the content of each type of inhibitor in the different sera and to find the simplest methods of destroying or reducing their activity.

RÉSUMÉ

L'étude des inhibiteurs de l'hémagglutination du virus grippal, dans le sérum d'un certain nombre d'animaux, a montré l'existence de deux types d'inhibiteurs :

a) l'inhibiteur de Chu, qui est en général thermolabile et présente une activité plus grande vis-à-vis des virus A non adaptés à la souris que vis-à-vis des virus adaptés.

b) l'inhibiteur de Francis, qui est thermostable et plus actif vis-à-vis des virus B chauffés que vis-à-vis de ceux qui n'ont pas été chauffés.

Des essais ont été faits pour évaluer sommairement la teneur des divers sérums en ces deux types d'inhibiteurs et pour trouver les méthodes les plus simples permettant de supprimer, ou du moins de réduire, leur activité.

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