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Effect of Di-Ethyl-Amino-Ethyl (DEAE)-Dextran on the plaque reduction and plaque inhibition tests with arboviruses

BY

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Summary — The addition of DEAE-dextran to agar overlay dimishes the titer of a neutralising serum in the plaque reduction and inhibition tests. This is most probably through neutralisation of agar inhibitors, which otherwise enhance the inhibitory effect of a serum.
As a consequence, when serum surveys are performed, using the plaque inhibition technique with agar, the estimation of the percentage of neutralizing sera may be too high.

In the neutralisation reaction with plaque producing viruses, three different variants of the technique are possible :

1. *Plaque neutralisation* : mixtures of virus and serum are incubated *in vitro,* residual infectivity being determined by plaque titration, either after a fixed time(usually 1 hour), or at several moments (kinetic neutralisation test).

2. *Plaque reduction* : monolayers of cells are infected with a standard dose of virus. After incubation they are overlaid with agar containing serial dilutions of test sera.

3. *Plaque inhibition* : cellmonolayers are infected with a standard dose of virus and later overlaid with normal agar overlay medium. Beads dipped in antisera are placed on the surface of the agar and the plates are incubated.

When the serum contains neutralising antibodies, plaque formation in an area surrounding the beads is inhibited (Porterfield, 1960).

For a serological survey on sera from Senegal (Pattyn *et al.,* 1965), we used filter paper disks which had been dipped in serum.

DEAE-dextran neutralises inhibitors in the agar overlay medium or influences virus adsorption on tissue culture cells, it thus may enhance plaque diameters and/or plaque numbers (Pattyn *et al.*, 1966, 1967). We therefore investigated the influence of DEAE-dextran on the plaque reduction and inhibition tests.

Material and methods

These were described in former papers from this laboratory (Pattyn, de Vleesschauwer, 1966, 1967).

Most experiments were performed with Sindbis large plaque virus and a corresponding antiserum prepared in rabbits.

In some experiments, Semliki Forest virus was used, as will be indicated. All results were recorded after 48 hours incubation under agar, unless otherwise indicated.

Results

In experiment 1, using Sindbis-1 virus, the influence of DEAE-dextran on plaque reduction was clearly shown :

EXPERIMENT 1

Influence of DEAE-dextran on Sindbis-1 plaque reduction by serum

	Inoculum	Overlay containing		Plaque numbers
		serum	DEAE-dextran	
A	virus	—	—	35 (*)
B	virus	—	25 mg/ml	38
C	virus	1/80	—	0
D	virus	1/80	25 mg/ml	45
E	virus + 25 mg D-dext.	—	—	39
F	virus + 25 mg D-dext.	1/40	—	0 (12**)
G	virus + 25 mg D-dext.	1/80	—	0 (20**)

(*) Mean number of plaques on 3 petridishes.
(**) Plaques appearing with 24 hours delay and small, vague.

When 25 mg of DEAE-dextran was added to the serum containing agar overlay medium (line D), plaque reduction was completely abolished. However when DEAE-dextran was mixed with the inoculum, the neutralising effect of the serum was maintained although (lines F and G) small, vague plaques appeared in small numbers after 24 hours further incubation.

That the effect of DEAE-dextran was not on the interaction between serum and virus, was shown in experiments 2 to 4.

In experiment 2, DEAE-dextran was added to the virus-serum mixture and preincubated at 37 °C for 60' before inoculation on the cell monolayer. DEAE-dextran did not influence the result.

EXPERIMENT 2

DEAE-dextran added to Sindbis-1 virus-serum mixture before inoculation

		Plaque numbers
A	virus + BGT	17
B	virus + serum 1/80	0
C	virus + serum 1/80 + 25 mg/D. D	0

In experiment 3, the neutralisation reaction was performed with and without DEAE-dextran on chick embryo tissue cultures in tubes, where agar can not interfere.

The serum titer versus 100 TCD 50 of Sindbis-1 virus was 1/320 either in the presence of 30 mcg/ml DEAE-dextran, or in its absence. The same serum neutralized two logs of virus at a dilution of 1/40, again DEAE-dextran was without effect.

In experiment 4, the order of mixing the components making up the overlay medium was varied : serum was mixed with DEAE-dextran before the addition of the agar, and dextran was mixed with agar before the addition of the serum. These variations did not influence the results.

That virus inhibitors in the agar enhance the plaque inhibition titer of a serum was further evidenced by using agar, agarose and carboxymethyl cellulose (CMC) as solidifying substances (experiment 5).

The reducing effect of the serum was indeed much less pronounced under agarose than under agar, but was almost unchanged under CMC, although in a previous study this substance was found to be almost free of virus inhibitors (Pattyn and de Vleesschauwer, 1967).

EXPERIMENT 5

Incorporation of neutralizing serum in overlay medium solidified with agar, agarose and CMC

Serum dilutions	Number of plaques under		
	agar	agarose	CMC
no serum	31	38	35
1 : 10	0	18 (*)	0
1 : 20	0	22 (*)	0
1 : 40	0	17 (*)	pp
1 : 80	0		

(*) = Small plaques compared with controls.
pp = pin point plaques.

In experiment 6, the DEAE-dextran concentrations were varied : higher concentrations influence higher amounts of antibody. A dose effect relationship is much less evident under CMC overlay.

EXPERIMENT 6

Addition of DEAE-dextran in various concentrations to the agar or CMC overlays containing neutralizing serum

DEAE-dextran added mg/ml	Serum 1/20 agar	Serum 1/40	
		agar	CMC
0	0	0	0
15	0	0	4
30	0	0	1
60	14	22	0
125	12	25	5
250	0		0
Controls without serum or DEAE-dextran	31		34

All the plaques appearing under serum containing overlays were always smaller and sometimes very vague, when compared with the controls.

In experiment 7, two series of plates were overlaid with agar or agarose medium containing different amounts of DEAE-dextran and neutralizing serum. From one series of plates, the inoculum was removed after the one hour adsorption period, from the other it was not.

The results show that, when the inoculum is not removed, the number of plaques is higher than when it is removed, however the relative differences between the controls receiving no DEAE-dextran and those receiving it, are identical in both series, the proportion of uninhibited virus remains identical.

EXPERIMENT 7

Effect of leaving or removing the inoculum on plaque number under agar and agarose overlay medium containing DEAE-dextran and serum

Serum	DEAE-dextran mg/ml	Inoculum removed	Inoculum not removed
		agar overlay medium	
0	0	39	55
1 : 40	125	33	47
1 : 80	125	34	52
		agarose overlay medium	
0	0	44	50
0	15	39	54
1 : 10	15	29 vague	41 vague
1 : 20	15	28 vague	36
1 : 40	15	37	41
1 : 80	15	47	51

In experiment 8, the actual implication of these findings on the plaque inhibition test using filterpaper discs was tested.

Three different concentrations of Sindbis-1 virus were inoculated on CETC in Petri dishes. After adsorption, these were overlaid with medium containing agar or agarose, and DEAE-dextran. After solidifying, filter paper discs, dipped in dilutions of an anti-Sindbis serum were deposited on the surface. Three days later the inhibition zones were measured.

EXPERIMENT 8

Titer of a rabbit anti Sindbis serum by plaque inhibition method on filterpaper discs on different overlay media

Dilution of virus inoculum	Agar DEAE-dextran	added	mg/ml	Agarose	
	0	30	125	0	15
0,5 10^{-4}	32 (*)	8	2	8	8
0,5 10^{-3}	32	16	4	4	8
10^{-3}	16	32	2	4	2

(*) = Reciprocal of serum dilution giving an inhibition zone of 2mm or more.

The serumtiter was profoundly influenced by the addition of DEAE-dextran to the agar overlay or by the use of agarose, and more so than by the use of varying amounts of virus.

All these observations were made with Sindbis — large plaque forming virus, which is very sensitive to agar inhibitors and to the plaque enhancing effect of DEAE-dextran (Pattyn and de Vleesschauwer, 1969). We therefore performed another experiment with Semliki Forest virus, which is much less sensitive to these factors (Pattyn and de Vleesschauwer, 1968).

Experiment 9 shows that the plaque inhibition of this virus by its antiserum is also influenced by the addition of DEAE-dextran to the agar overlay.

EXPERIMENT 9

Influence of DEAE-dextran on the plaque reduction test with Semliki Forest virus

Serum dilution in overlay	DEAE-dextran concentration in overlay mg/ml		
	0	15	62.5
0	39	39	34
1 : 40	0	11 very vague	25
1 : 80	25 vague	23 vague	25

This was also the case with West Nile virus *(experiment 10)* :

EXPERIMENT 10

Effect of DEAE-dextran on plaque reduction test with West Nile virus

Serum dilutions	DEAE-dextran added to overlay medium		
	0	15	89
0	118	102	89
1 : 10	0	0	0
1 : 20	0	0	66
1 : 40	0	0	78
1 : 80	60	45	88

0.25 mg/ml cortisol was incorporated in all overlays.

In a last experiment, the influence of DEAE-dextran on the titer of an interferon preparation was essayed.

Interferon dilutions were added to CETC in petridishes, 24 hours before virus inoculation, followed by addition of overlay medium with and without varying amounts of DEAE-dextran.

The interferon titer was not influenced by the presence of DEAE-dextran in the overlay medium.

Discussion

Since DEAE-dextran enhances plaque production by arboviruses under agar (Pattyn and de Vleesschauwer 1967,1969), it could be foreseen that this substance would also influence the titer of a neutralizing serum in the plaque reduction and inhibition tests.

The inhibitors normally present in agar actually enhance the inhibitory activity of a neutralizing serum. Through neutralisation of these inhibitors, DEAE-dextran diminishes the activity of an antiserum in the plaque inhibition and reduction tests.

The neutralisation itself is not influenced by DEAE-dextran, as is shown by the results of experiments 2, 3 and 4.

The titer of an interferon preparation also was not altered when the test was done as described. Indeed interferon acts intracellularly, and was added 24 hours before the DEAE-dextran.

The practical implication of this is that by using the plaque inhibition technique, titers of neutralizing sera may be enhanced, and in serum surveys, the number of neutralizing sera may be increased.

Samenvatting. — **Beinvloeding door DEAE-dextran van plaque reductie en plaque inhibitie testen voor arbovirussen.**

Wordt bij de plaque reductie en plaque inhibitie methodes DEAE-dextran aan de agar toegevoegd, dan verlaagt de titer van een neutraliserend serum. Dit effekt is het gevolg van de neutralisatie door DEAE-dextran van agar inhibitoren, die bij afwezigheid van dit produkt, de remmende werking van het serum verhogen.

Bijgevolg kan het percentage neutraliserende sera te hoog uitvallen indien bij serologisch massaonderzoek de plaque inhibitie test met agar wordt gebruikt.

Résumé. — **Effet du DEAE-dextran sur les tests de réduction et d'inhibition de plages pour les arbovirus.**

Dans les techniques de réduction et d'inhibition des plages, l'addition de DEAE-dextran au milieu gélosé diminue le titre d'un sérum neutralisant. Cet effet est très probablement le résultat de la neutralisation d'inhibiteurs présents dans la gélose et qui, en l'absence de DEAE-dextran renforcent l'action inhibitrice du sérum.

Par conséquent, lors d'enquêtes sérologiques utilisant la technique de l'inhibition des plages sous gélose, l'estimation du pourcentage de sérums neutralisants peut être trop élevée.

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