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The effect of kaolin and acetone-ether treatment on HI antibody titres to arboviruses in chicken serum

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INTRODUCTION

The demonstration of haemagglutination with a number of arboviruses (Sabin & Buescher, 1950; Macdonald, 1952; Chanock & Sabin, 1953) provided the relatively simple haemagglutination inhibition (HI) test for detection of antibody. The chief disadvantage of HI tests has been the presensce of arbovirus non-specific haemagglutination inhibitor (NSI) which occur in human and animal sera. For the removal of the NSI in sera, acetone-ether extraction and kaolin adsorption methods were proposed (Porterfiied, 1954; Clarke & Casals, 1958). Kaolin has also been used to adsorb non-specific inhibitors of adenovirus, coxsackeivirus, echovirus, measles virus and reovirus (Schmidt & Lennette, 1965). It has been suggested however that treatment with kaolin not only removed non-specific inhibitors but also reduced the titre of specific antibody (Karpov & Selezneva, 1964; Schmidt, Dennis, Hagens & Lennette, 1962).

The following experiment was undertaken to determine the effect of kaolin treatment and acetone-ether extraction on HI antibody titres to MVE and Sindbis viruses in order to select a method which would eliminate non-specific inhibitor without simultaneously removing antibody.

MATERIALS AND METHODS

(1) Virus strains

Sindbis (MRM 39): Eighth suckling mouse brain passage of virus which was isolated originally from *Culex annulirostris* at Mitchell River in 1960 was used (Doherty *et al.*, 1963).

Murraly Valley encephalitis MVE, MRM 66): This strain was isolated originally from *C. annulirostris* at Mitchell River in 1960 and had undergone seven mouse brain passages (Doherty *et al.*, 1963).

(2) Preparation of virus infectious materials

Two to six days old mice were inoculated by the cerebral route, the dose usually being 0.02 *ml.* of a 10 dilution of stock infectious suckling mouse brain. Conditions were adjusted so that the brains could be harvested at a time when some of the mice were beginning to die and the rest appeared sick from the infection. The animals were partially exsanguinated by cutting through the chest wall with small scissors, after which the brains were removed and transferred to small, weighed glass bottles held in a bath of dry ice. The frozen mouse brains were homogenized in a homogenizer with nine volumes of tris Hank's solution, followed by centrifugation for 30 minutes at 8,400 G in the cold. The supernatant fluid of that homogenate was used as the ten percent virus suspension.

(3) Preparation of chicken immune sera

Twelve chickens were divided into three groups of four. One group of chickens was inoculated with 10^5 mouse LD_{50} of Sindbis virus suspension, and the second group with 10^5 LD_{50} of MVE virus suspension, both intramuscularly. A third group was used as an uninoculated control group.

Chickens in group one were bled from the heart at one week, three weeks, five weeks, six weeks, 11 weeks and 16 weeks after inoculation. Chickens in the second group were bled from the heart at two weeks, three weeks, five weeks and six weeks after inoculation. The control group of chickens was bled from the heart at one weeks, two weeks, three weeks, five weeks, six

weeks, 11 weeks and 16 weeks after inoculation of the viruses in the other two groups of chickens. The sera was stored at −20°C after collection.

(4) Serological test: Haemagglutination-inhibition (HI) test.

The technique of this test was adapted from the method of Clarke & Casals (1958), using microtechnique (Sever, 1962).

(5) Zonal centrifugation of chicken immune sera

Sixteen chicken antisera to Sindbis virus and ten chicken antisera to MVE virus were subjected to zonal centrifugation. Each serum was divided into six parts, and grouped into three groups of two. One group of sera was adsorbed with kaolin, another group was extracted with acetone-ether, and the third group was used as untreated control sera.

Table 1. Effect of kaolin and acetone-ether treatment on Sindbis virus HI antibody titres in chicken sera

Serum No.	Titre of HI antibody		
	Untreated	Kaolin	Acetone-ether
1	1280	640	640
2	1280	640	640
3	320	160	160
4	320	160	160
5	80	20	40
6	80	40	20
7	160	160	160
8	160	160	160
9	80	80	80
10	80	80	80
11	160	160	160
12	160	160	160
13	80	40	40
14	80	40	20
15	80	40	40
16	80	20	40

* Reciprocal dilution of serum fractions

Note: Non-specific inhibitors were removed by zonal centrifugation.

A 5.4*ml.* amount of the sera was subjected to zonal centrifugation in a linear ten to 40 percent sucrose gradient. After centrifugation, each serum was fractionated into 15 equal fractions. The top four fractions were discarded because non-specific inhibitors were found to be concentrated in these fractions (Chung, 1969).

In one centrifuged serum sample of each group, the HI antibody titre of each fraction, and the HI titre of the 11 fractions combined (pooled serum) was determined. The other sample was divided into two parts, one part of the four bottom fractions (IgM), and the other of fractions five to 11 (IgG), and the HI antibody titre of each part was determined. The normal chicken sera obtained from the uninoculated control group were fractionated as were the antisera to MVE and Sindbis viruses, after which the fractions were examined for non-specific reaction against MVE and Sindbis viruses.

EXPERIMENTAL RESULTS

(1) Effect of kaolin tratment on Sindbis and MVE virus antibody levels in chicken immune sera:

In the 16 Sinbdis virus immune sara, HI antibody titres of two sera (12.5%) were decreased four-fold, eight sera (50%) were decreased two-fold, and six sara (37.5%) were not affected by the adsorption with kaolin in the pooled sera (Table 1). Ten treated and untreated sera had an IgM type antibody, of which two (20%) showed a decrease from 1:20 to below the level of detection, four (40%) showed a decrease from 1:10 to below the level of detection, two (20%) showed a four-fold decrease and two (20%) showed a two-fold decrease in titre (Table 2). In the IgG fraction, nine sera(56%) showed a two-fold decrease and titres were unchanged in seven sera (44%) (Table 2).

In the ten MVE virus immune sera, HI antibody titres in the pooled sera of eight (80%) showed a two-fold decrease while the other two (20%) remained unaffected (Table 3). In the IgM fraction, two sera (20%) showed a decrease from 1:40 to below the level of detection, two (20%) showed a decrease from 1:20 to below the level of detection, three (30%) showed a four-fold decrease, two (20%) showed a two-fold decrease and one (10%) was unaffected (Table 4). In the IgG fraction, five sera (50%) showed a two-fold decrease while titre in the other five sera (50%) were unaffected (Table 4).

(2) Effect of acetone-ether extraction on Sindbis and MVE virus antibody levels in chicken immune sera:

In the 16 Sindbis virus immune sera, HI antibody

Table 2. Effect of kaolin and acetone-ether extraction on two immunoglobulin types of HI antibody to Sindbis virus.

Week++	Serum No.	HI titre of IgM and IgG+					
		Untreated		Kaolin		Acetone-ether	
		IgM	IgG	IgM	IgG	IgM	IgG
1	1	320	160	160	80	160	80
	2	320	160	160	80	160	80
3	3	80	160	20	80	40	80
	4	80	160	20	80	40	80
	5	10	20	<10	10	10	10
	6	10	20	<10	10	10	10
5	7	10	160	<10	80	10	160
	8	10	160	<10	80	10	160
6	9	20	40	<10	40	<10	40
	10	20	40	<10	40	<10	40
11	11	<10	160	<10	160	<10	80
	12	<10	160	<10	160	<10	80
16	13	<10	40	<10	40	<10	40
	14	<10	40	<10	20	<10	20
	15	<10	40	<10	40	<10	20
	16	<10	40	<10	40	<10	20

+ Reciprocal dilution of the serum fractions

++ Weeks following inoculation of the virus

Table 3. Effect of kaolin and acetone-ether treatment on MVE virus antibody titres in chicken sera

Serum No.	Titre of HI antibody+		
	Untreated	Kaolin	Acetone-ether
1	640	320	160
2	640	320	160
3	320	160	160
4	320	160	160
5	320	160	160
6	320	160	160
7	160	80	160
8	160	160	160
9	160	80	160
10	190	160	80

+ Reciprocal dilution of the serum fractions

Note: Non-specific inhibitors were removed by zonal centrifugation.

titres of two sera (12.5%) were decreased four-fold, eight (50%) were decreased two-fold, and six (37.5%) were unaffected by extraction with accetone-ether in the pooled sera (Table 1). Ten treated and untreated sera had an IgM type antibody, of which two sera (20%) showed a decrease from 1:20 to below the level a of detection, four (40%) showed a two-fold decrease and

Table 4. Effect of kaolin and acetone-ether extraction on two immunoglobulin types of HI antibody to MVE virus

week++	Serum No.	HI titre of IgM and IgG+					
		Untreated		Kaolin		Acetone-ether	
		IgM	IgG	IgM	IgG	IgM	IgG
2	1	320	80	90	80	40	40
	2	320	80	80	70	40	40
3	3	40	160	<10	80	20	80
	4	40	160	10	80	20	80
5	5	20	320	20	260	20	160
	6	20	320	<10	160	20	320
6	7	40	80	20	80	40	80
	8	40	80	<10	80	40	80
	9	20	80	<10	40	20	80
	10	20	80	10	80	20	40

+ Reciprocal dilution of the serum fractions

++ Weeks following inoculation of the virus

titres were unchanged in four (40%) of the sera (Table 2). In the IgG fractions, 11 sera (69%) showed a two-fold decrease and titres were unchanged in five (31%) (Table 1).

Table 5. Summary of the effect on HI antibody of IgM and IgG type of treatment with kaolin and acetone-ether

Virus	Class of antibody	Percentage of sera tested in which serum titer of HI antibody declined		Total sera tested
		Kaolin	Acetone-ether	
Sindbis	1gM	100%(10)+	60%(6)+	10
	1gG	56 (9)	69 (11)	16
MVE	1gM	90% (8)	40% (4)	10
	1gG	50 (5)	60 (6)	10

( )+ No. of sera

In the ten MVE virus immune sera, HI antibody titres in the pooled sera of two (20%) showed a four-fold decrease, and five (50%) showed a two-fold decrease, while the other three (30%) remained unaffected (Table 3). In the IgM fraction, two sera (20%) showed an eight-fold decrease, and titres were unchanged in six (60%). In the IgG fraction, six sera (60%) showed a two-fold decrease while titres in the other four sera (40%) were unaffected (Table 4).

Overall, in the IgM fraction of Sindbis virus antisera, kaolin adsorption decreased the HI antibody titre of all sera tested, and acetone-ether extraction decreased the titre of 60 percent of the sera tested. In the IgG fraction of Sindbis virus antisera, Kaolin adsorption decreased the HI antibody titre of 56 percent of the tested sera, and acetone-ether extraction decreased the titre of 69 percent of the sera tested.

In the IgM fraction of MVE virus antisera, kaolin adsorption decreased the HI antibody titre of 90 percent of the tested sera, and acetone-ether extraction decreased the titre of 40 percent of the sera tested. In the IgG fraction, kaolin adsorption decreased the HI antibody titre of 50 percent of the sera tested, and acetone-ether extraction decreased the titre of 60 percent of the tested (Table 5).

DISCUSSION

Both IgM and IgG type HI antibody titres to Sindbis and MVE viruses in chicken sera were effected by kaolin adsorption and acetone-ether extraction. The surprising was the greater loss of titre in the IgG fraction following acetone-ether extraction than that following kaolin adsorption for both Sindbis and MVE virus antisera, while in the IgM fraction of Sindis and MVE virus antisera the HI titres were more affected by treatment with kaolin than acetone-ether extraction. The effect of kaolin adsorption is similar to that reported by Gaidamovich, Mekler & Kahaltayeva (1967) who found that kaolin adsorption of similar fraction of rabbit antiserum to arbovirus to resulted in complete removal of macroglobulin antibody (IgM), and a two-fold reduction of titre in the IgG fraction. In contrast to this, Mann, Rossen, Lehrich & Kasel (1967) who examined the effect of kaolin adsorption on *Salmonella typhosa* antibody levels in human sera, and the effect of kaolin and heparin-$MnCl_2$ treatment on the immunoglobulin levels to reoviruses in human antisera, found that kaolin adsorption resulted in a four-fold or greater loss of antibody activity in all fractions and that antibodies in the IgG fraction seemed to be affected more than antibodies in the IgM fraction. They also found that heparin-$MnCl_2$ treatment of human antisera to reoviruses removed less than 10 percent of each immunoglobulin class, but that kaolin adsorption resulted in a significant loss of each immunoglobulin fraction, namely IgG, IgA and IgM.

In the results of present study and those reported by Mann *et al* (1967) the significant loss of each immunoglobulin fraction IgG and IgM by kaolin adsorption was similar, but Mann and his co-workers reported that IgG fraction was affected more than the IgM fraction after kaolin adsorption, whereas in the present report the converse was true.

SUMMARY

The experiment was undertaken to determine the effect of kaolin treatment and acetone-ether extraction on HI antibody titres to MVE and Sindbis viruses in chicken serum.

It has been found that both kaolin adsorption and acetone-ether extraction not only removed the NSI but also reduced the titre of specific IgM and IgG type HI antibodies to MVE and Sindbis viruses in chicken serum.

With IgM type antibody in both Sindbis and MVE virus antisera, the HI titres were more affected by adsorption with kaolin than by acetone-ether extraction. However, with IgG type antibody, the HI titre seemed to be more affected by acetone-ether extraction than by kaolin adsorption.

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Kaolin 및 Acetone-ether 처리가 抗腦炎病毒 鷄血淸의 특이 항체에 미치는 영향

家畜衛生研究所

鄭 榮 錫

닭 血淸속에 存在하는 腦炎病毒에 對한 非特異性 억제 物質을 除去하기 위하여 Kaolin 또는 Acetone-ether로서 그 血淸을 처리 하였을때 그 처리가 非特異性 억제物質 뿐만 아니라 特異한 抗體의 力價에 對한 영향의 有無를 시험하였다.

Kaolin 및 Acetone-ether 처리는 확실히 特異抗體의 HI 力價의 減少를 가져왔다.

IgM type 항체는 Kaolin 처리에 의해서 그리고 IgG type 항체는 Acetone-ether 처리에 의해서 더 큰 抗體力價의 減少를 가지 왔다.