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The nature of non-specific inhibitor and natural agglutinin for goose erythrocytes in chicken serum

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INTRODUCTION

The investigation of the non-specific inhibitors (NSI) to arboviruses and natural agglutinin (HA) to goose erythrocytes in chicken serum is important in view of the extensive use of haemagglutination inhibition (HI) procedures for diagnosis of virus infections.

The non-specific inhibitory activity of human and animal sera on arbovirus haemagglutination (HA) is well known (Sabin and Buescher, 1950; Chanock and sabin, 1953a; Clarke and Casals, 1958; Holden, LaMotte and Shriner, 1965). Salminen (1962) reported that group A and group B arbovirus antigens shared the same NSI, but Porterfield and Rowe (1960) suggested that there was a sharp distinction of the NSI activity between the two groups.

The NSI in serum have been shown to be thermostable (Holden, LaMotte and Shriner, 1965; Verani and Gresikova, 1966), and are lipid or lipoprotein in nature(Salminen, 1960a). Porterfield and Rowe (1960) showed that purified serum lipoproteins were inhibitory, and Salminen, Renkonen and Renkonen (1960a, b) showed that mixtures of purified serum lipids were inhibitory.

Nicoli and Acker (1965) separated human serum into natural molecular groupings and showed that the inhibitory action was due to alpha-and beta-lipoproteins. Salminen (1962) suggested that the inhibitory activity of normal human sera was due to a mixture of two lipids, one of which was free cholesterol, the other was either a phospholipid or a free fatty acid. Hana, Styke and Schramek (1963) studied the molecular size of specific anti-influenza haemagglutinin obtained by separation in Sephadex G200 and concluded that the NSI and NA for rooster erythrocytes in bovine serum showed a similarity to macroglobulin (IgM).

For the removal of the NSI to arbovirus haemagglutinins several methods have been employed: extraction with acetone (Chanock and Sabin, 1953a; Clarke and Casals, 1958), with acetone and ether (porterfield, 1954a), with chloroform (sabin and Buescher, 1950), by Seitz filtration (Casals and Brown, 1954), and by adsorption with bentonite (Clarke and Casals, 1955) or kaolin (Clarke and Casals, 1958).

As the NSI were reported to be lipid or lipoprotein in nature, and therefore less dense than proteins, Osterreith (1967) considered it might be possible to remove the NSI by ultracentrifugation.

Although acetone has been used for the removal of the NSI, Holden, LaMotte and Shriner (1965) and Holden, Muth and Shriner (1966) demonstrated the presence of acetone-insoluble NSI in sera of adult female chickens.

Sera of animals and humans contain naturally occurring agglutinins for goose erythrocytes. These NA can be removed by adsorption with erythrocytes prior to use in tests (Clarke and Casals, 1958). The NA are also heat labile as shown by Landsteiner (1945).

The following experiments were performed to provide a basis for HI tests utilizing chicken serum: (a) the affect of kaolin adsorption and acetone-ether extraction on the NSI before and after heating at various temperatures, (b) an examination of the physical properties of NA and NSI by gel filtration and zonal centrifugation, and (c) methods of removing the NA.

MATERIALS and METHODS

(a) **Viruses (Doherty et al, 1963)**

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

Getah (N544). This strain was isolated originally from *Anopheles amictus amictus* at Normanton in 1961 and has undergone seven mouse brain passages.

Ross River (T48). Sixth mouse brain passage of the strain isolated originally from *C. annulirostris* at Mitchell River in 1960 was used.

Murray Valley encephalitis (MVE, MRM66). This strain was isolated originally from *C. annulirostris* at Mitchell River in 1960 and had undergone seven mouse brain passages.

Stratford (C338). Sixth mouse brain passage of the strain which was isolated originally from *Aedes vigilax* at Cairns in 1961 was used.

Edge Hill (C281). This strain was isolated originally from *Aedes vigilax* at Cairns in 1961 and had undergone seven mouse brain passages.

Kunjin (MRM 16). Sixth mouse brain passages of the virus which was isolated originally from *C. annulirostris* at Mitchell River in 1961 was used.

Kokobera (MRM 32). This strain was isolated originally from *C. annulirostris* at Mitchell River in 1960 and had undergone eight mouse brain passages.

(b) **Virus infectious materials**

Two to six days old mice were inoculated by the cerebral route, the dose usually being 0.03 ml. of a 10^{-2} 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 at 8,400G for 30 minutes in the cold. The supernatant fluid of the homogenate was used as a ten per cent virus suspension.

c) **Serological procedures**

Serological tests were carried out in haemagglutinati (HA) plates. The "macro" test was carried out plates with 1.2 ml. cavities, using a unit volum 0.2 ml. Some of the early HI tests, all HA titratio and any tests where accurate end point determinati was required were done in these plates. Reagents f the tests were distributed into the cavities with a automatic syringe.

Serial dilutions were made with a short paste pipette attached to the barrel of a "Cornwall" aut matic syringe (with the three-way valve removed) b means of a short rubber tube forced over a hypoderm needle from which the shaft had been removed. Th allowed serial dilutions to be carried out more rapid and with greater reproducibility than had been po sible with a graduated pipette.

The "micro" test was carried out with microtitratic apparatus(Takatsky, 1955; Sever, 1962). This consis of "loops" calibrated to hold either 0.025 or 0.05 n by capillarity and pipettes calibrated to deliver dro of 0.025 ml. Both perspex and disposable plastic plate were used, although the latter were preferred as the allowed better heat distribution. Plates with a "V bottom were used for agglutination tests (HA an HI), and "U" bottem plates were used for the C test.

After use the plates were decontaminated overnig in a detergent solution containing hypochlorite ("Div ersal CX" Diversey A/Asia Pty. Ltd., N.S.W.), an washed in an automatic washing machine, whic included a distilled water rinse. These were washe as decribed and re-used until they became badl scratched or marked.

1) *Haemagglutination-inhibition test (HI)*

The technique of this test was adapted from th method of Clarke and Casals (1958), using micro technique (Sever, 1962).

Preparation of antigen: The method was adapte from the method of Clarke and Casals (1958). Th infected suckling mouse brain or blood was homoge nized with four volumes of a chilled nine percen aqueous solution of sucrose. The homogenate wa added dropwise, with stirring, to 15 volumes o

chilled acetone.

The mixture was shaken and allowed to stand at 4°C for 15 minutes or 20 minutes. The milky supernatant fluid was discarded. The extraction with acetone was repeated as before and then a volume of fresh acetone equal to that originally used was added to the bottle and the sediment was suspended by vigorous shaking, and the preparation was held at 4°C for 60 minutes to dehydrate the sediment. After sufficient time the sediment was reduced to a fine suspension by use of a thick glass rod. The bottles were then centrifuged at 500 G, for ten minutes the supernatant was discarded, the sediment was dried with freezine using a "Hyvac" oil pump.

To the dry powder, a volume of borate saline, pH 9.0 (BS), was added which is equal to four-tenth of the total volume of homogenate used. The preparation was allowed to stand at 4°C for 16 to 18 hours. It was then centrifuged for 30 minutes at 8,400 G. The supernatant fluid was the antigen. It was dispensed in one ml. ampoules and stored at -50°C.

(d) Chicken sera

The sera were obtained from laboratory-reared chickens by heart puncture, and stored at -20°C.

1). Fifty sera were obtained from normal chickens more than 15 weeks old, and used to examine: (i) the effect of heating at 60°C, 80°C and 100°C on the NSI, (ii) the effect of acetone-ether extraction and kaolin adsorption on the NSI to Sindbis and MVE viruses. Each serum was divided into 42 parts, 21 for testing of the NSI activity against Sindbis virus (table 4), and 21 for testing of the NSI activity against MVE viruse (table 5). The sera were examined before and after heating.

2) Sera were obtained from 50 normal chickens more than 10 weeks of age and used to examine: (i) the activity of the NSI to both group A and group B arboviruses (table 1), and (ii) to determine the effect of pH on the NA (table 6).

3) Five or six normal chicken sera of seven different age groups, namely five, seven, ten, 12, 16, 24 and 28 weeks, were used to determine the influence of age on the NSI to group A and group B arboviruses (table 21).

4) Chicken immune sera were produced in six ten weeks old chickens each inoculated intramuscularly with 10^6 mouse LD 50 of MVE virus. Blood was collected at 14 days after inoculation. Each immune serum was divided into two parts. One part was diluted to two fold and four fold in normal chicken serum, after which each dilution was adsorbed with kaolin and ten percent goose erythrocytes and used to determine the effect of heat on HI antibody, and the NA without prior dilution and adsorption with goose erythrocytes (table 8).

5) Seventy normal chicken sera were examined to determine the effect of acetone-ether and kaolin treatment on the NA. Each serum was divided into three parts for treatment with either kaolin or acetone-ether, and one part was left as an untreated control. (Table 7).

(e) Titration of non-specific inhibitor

Non-specific inhibitors to viral haemagglutinin in the sera were determined by diluting serum 1:10 in borate saline at pH 9.0 (BS pH 9.0), and each dilution was adsorbed with ten percent goose erythrocytes prior to titration. The titration technique was adopted from that of Clarke and Casals (1958), using micro technique (Sever, 1962). Viral antigens were extracted from infected suckling mouse brains by the sucrose acetone method of Clarke and Casals (1958). To serial dilutions of serum eight units of antigen were added and incubated overnight at 9°C. Goose erythrocytes were added, and agglutination at the optimum pH for each virus was allowed to take place at room temperature. The titres of NSI were expressed as the reciprocal of the highest serum dilution showing complete HI.

(f) Titration of natural agglutinin

Natural agglutinin to goose erythrocytes in chicken sera was determined by making serial two fold dilution of the serum in BS pH 9.0 using an initial 1 : 10 dilution of serum in BS pH 9.0 and the micro technique of Sever (1962). Ten percent goose erythrocytes were added in adjusting diluent of appropriate pH to each dilution, and the plates were allowed to stand at room temperature.

(g) Heating of sera

To determine the effect of heat on the NSI the sera were placed in 5/8″ glass centrifuge tubes and heated

at 60°C, 80°C and 100°C in constant temperature water baths. At 100°C sera were diluted 1 : 2 in 0.15 M NaCl before heating.

To determine the effect of heat on the NA and HI antibody, the sera were placed in glass centrifuge tubes and heated at 56°C, 60°C, 70°C, 80°C and 90°C in constant temperature water baths.

(h) Zonal centrifugation

For investigation of the NSI, three normal chicken sera, and one immune chicken serum obtained from a chicken infected with MVE virus, were used. A 0.4m*l* volume of each serum was subjected to zonal centrifugation in a linear ten to 40 percent sucrose gradient.

For investigation of the NA, five normal chicken sera were pooled and 0.4m*l* of the pooled serum was subjected to zonal centrifugation in identical gradients. After centrifugation the gradients were divided into 15 equal fractions.

(i) Gel filtration

For fractionation with Sephadex G200 to identify the location of the NSI and NA, sera from five normal chickens were pooled. One m*l* of the serum was used in each run, and 14 equal fractions were made from the eluates.

RESULTS

(1) Activity of non-specific inhibitor to different groups of arboviruses

Non-specific inhibitors were demonstrated in chicken sera to all the test viruses. Of 50 normal sera examined, 43 contained NSI to the group A arboviruses in titres ranging from 20 to 1280, and all contained NSI against the group B arboviruses in titres ranging from 20 to 5120 (table 1).

(2) Influence of age on non-specific inhibitors in serum

No variation in the NSI titre against the group A arboviruses was observed with age. In contrast, the NSI titres against the group B arboviruses were found to increase with age, especially those active against MVE, Edge Hill and Kunjin viruses (tables 2, 3).

(3) Effect of heat, kaolin and acetone-ether on non-specific inhibitors

Kaolin adsorption and acetone-ether extraction significantly decreased the number of sera showing the NSI activity against Sindbis and MVE viruses (tables 4, 5) when sera were examined before heating.

The affect of heating the sera at 80°C and 100°C was to increase the NSI against Sindbis and MVE viruses so that the percentage of sera reacting with the two antigens tested was almost 100 percent. The distribution of the NSI in the 50 examined sera can be seen in tables 4 and 5.

Kaolin and acetone-ether treatment of sera after heating at 80°C and 100°C was less successful than was the same treatment of the unheated sera.

(4) Investigation of non-specific inhibitor and natural agglutinin by zonal centrifugation

Three peaks were obtained by protein analysis of the fractions taken from density gradient tubes. All of the NSI activity against MVE virus in normal chicken sera was concentrated in the top three fractions. In fractions collected from immune serum, however, all of the NSI activity was concentrated in the top four fractions, and the HI antibody appeared in the top fraction and gradually decreased in the preceeding fractions (Figures 2a, b).

The NA was shown to have the properties of IgM by zonal centrifugation (Figure 1a).

(5) Investigation of non-specific inhibitor and natural agglutinin by gel filtration

The NSI to MVE virus occurred mainly in the first peak (IgM). However, a small amount of the NSI was scattered in a part of the second peak (IgG) Figure 2c).

The NA was shown to have the properties of IgM by passing through a Sephadex G200 column (Figure 1b).

(6) Effect of pH on natural agglutinin

Fifty chicken sera were divided into four groups and tested at four different pH levels: 6.5, 6.7, 6.9 and 7.1. All the test sera contained NA for goose erythrocytes. Titres ranged from 10 to 640, but most of them were distributed in the range of 10 to 80. As the NA appeared to be unaffected by pH (Table 6), the adjusting diluent of pH 6.7 was used for the following examination of the NA in chicken serum.

(7) Effect of acetone-ether and kaolin treatment on natural agglutinin

The NA was partly destroyed by acetone-ether extraction but not by kaolin adsorption (Table 7).

(8) Heat stability of natural agglutinin and HI antibody

Heating at 70°C for 10 minutes was the most effective procedure found for inactivation of the NA. However, the HI antibody was stable for 10 minutes at 70°C (Table 8). This was confirmed by an examination of five other immune sera.

(9) Removal of natural agglutinin by adsorption with goose erythrocytes

Three sera were tested for removal of agglutinins using ten and twenty percent suspensions of goose erythrocytes. A series of adsorptions was carried out at pH 9.0, and the sera were tested for agglutinins at pH 6.7.

Original titres of the NA of these sera were relatively high, but the NA was adsorbed comppetely after treatment with ten percent goose erythrocytes for ten minutes (table 9).

DISCUSSION

The natural agglutinins (NA) for goose erythrocytes in chicken serum were found to possess the properties of IgM immunoglobulins by gel filtration through Sephadex G200 and by zonal centrifugation (Figures 1a, b). These results are in accordance with those of Gaidamovich, Mekler & Kahaltayeva (1967), and Mekler, Kahaltayeva & Gaidamovich (1966) who found that the NA in rabbit serum occurred in the 19S (IgM) fraction obtained by gel filtration through Sephadex G200.

The NA was partially destroyed by extraction with acetone-ether and was efficiently adsorbed with ten percent goose erythrocytes at 9°C for 15 minutes, but no affect was observed by using several adjusting buffers of varying pH(tables 6,7,8). This is in contrast to a report by Maydat & Demarchi (1965) that the NA for goose erythrocytes in bovine serum was not efficiently adsorbed by goose erythrocytes under the conditions used for the routine adsorption prior to the HI test.

In the present study, acetone-ether extraction significantly decreased the number of chicken sera showing the NSI activity, but some NSI still remained in the sera (tables 4, 5). Doherty, Gorman, Whitehead & Carley (1966) also found that sera from many domestic fowls still retained the NSI to group A arbovirus after acetone-ether extraction, but not after kaolin adsorption. Holden, LaMotte & Shriner (1965) also showed the presence of acetone insoluble NSI in chicken sera, but not in human, horse and other mammalian sera examined. No significant difference could be demonstrated between acetone-extraction and kaolin adsorption on the rate of removal of the NSI in chicken sera in the present study (Tables 4,5). In contrast to this result, Doherty, Carley & Lee (1959) showed that NSI to arboviruses in human sera were not completely removed by kaolin treatment, the kaolin treated sera exhibiting titres usually two dilutions higher than those in sera treated with acetone. Sanderson (1967) showed that acetone ether extraction was more efficient in removing NSI in bovine sera than was kaolin adsorption.

The NSI in chicken serum was active against both group A and group B arboviruses but the effect was greater with the grorp B arboviruses (Table 1). This result was similar to that of porterfield & Rowe (1960) who showed that lipoprotein fractions from human serum, phospholipid extracts from human erythrocytes, and egg lecithin inhibited haemagglutination by group B, but not by group A arboviruses. In contrast to these reports. Salminen (1962) showed that neither lecithin nor palmitic acid inhibited HA by arboviruses, but that mixtures of these compounds with cholesterol inhibited HA by both group A and group B arboviruses. In the present study, some selectivity of these NSI to different strains of group A and group B arboviruses was demonstrated (Table 1). Similar result was obtained by Holden, LaMotte & Shriner (1965) who reported a marked selectivity of the NSI in chicken serum to different strains of group A arboviruses.

Heating the chicken sera at 80°C and 100°C for 10 and 30 minutes did not alter the percentage of sera containing NSI and had the adverse affect of increasing the titre of NSI (Tables 4,5). The increased NSI activity after heating might be due to denaturation of the inhibitor which could result in increased exposure of sites capable of combining with viral haem-

agglutinins. Verani & Gresikova (1966) also showed that thermostable inhibitors in normal human serumto group A and group B arbovirus haemagglutinins were still present after heating at 56°C for 30 minutes, and that this activity was enhanced four to eight fold by heating at 100°C for 30 minutes. This indicated that were two kinds of NSI in human serum, namely thermostable NSI and thermolabile NSI. Mekler, Kahaltayeva & Gaidamovich (1966) similarly showed that thermostable NSI to arboviruses in normal rabbit serum was not affected by heating at 56°C for 30 minutes, but increased two fold after heating at 100°C for two to three minutes. Holden, LaMotte & Shriner (1965) also found the NSI in chicken serum to be thermostable. Schmidt, Dennis, Hoffman & Lennette (1964) also reported that heating human serum at 56°C for 30 minutes, or 60°C for 30 minutes, had no effect on the NSI titres to ECHO virus and reovirus haemagglutination, but that heating at 80°C significantly increased the inhibitory activity of the sera. In contrast to these reports, Gresikova & Sekeyova (1967) stated that a thermostable NSI to group A and group B arboviruses could not be detected in goat and chicken serum.

After heating, acetone-ether extraction and kaolin adsorption of the sera failed to remove completely the NSI for group A and group B arboviruses, but extraction with acetone-ether was a little more effective than kaolin adsorption in these circumstances (Table 4, 5). In contrast Gresikova & Sekeyova (1967) found that after heating at 56°C and 100°C the NSI in bovine serum could be removed by acetone-ether extraction, and Verani & Gresikova (1966) also showed that the NSI in human serum could be removed by acetone-ether extraction after heating the serum at 100°C for 30 minutes.

No reason for the difficulty in removing the NSI in heated chicken sera can be presented.

Mann, Rossen, Lehrich & Kasel (1967) examined the NSI in human serum to reovirus and concluded that the NSI was found only in those fractions which contained betalipoprotein by gel filtration with Sephadex G 200 (mainly in the first peak and by diffusion to a part of second peak) and suggested that the NSI of reovirus may be betalipoprotein or a substance associated with beta-lipoprotein. Salminen (1960a), Porterfield & Rowe (1960), and Nicoli & Acker (1965) suggested that the NSI to arboviruses in human and animal sera were probably also lipids or lipoprotein in nature. Five types of lipids, namely, unesterified cholesterol, glycerides, cholesteryl esters, phospholipids and unesterified fatty acid are present in human serum as constituents of lipoproteins. These lipoproteins exist in several broad spectra within the density range of 0.93 to 1.16gm/ml. Thus, the lipoprotein classes may be isolated by floatation in the preparative ultracentrifuge. Ultracentrifugation at 40,000rpm for 10 hours is sufficient for isolation of the low density lipoproteins which are concentrated in approximately the top 0.5ml of the tubes (Lindgren & Nicholas, 1960). Wallenius, Trautman, Kunkel & Franklin (1957) also showed that the same preparation methods provide lipoprotein free normal human serum. In the present study, following fractionation of chicken serum by gel filtration, the NSI was mostly found in the first peak (IgM) and diffused to a part of the second peak (IgG) (Figure 2c) as all of the NSI activities were found on the top of the centrifuged tubes (Figures 3a, b). Those properties of large molecular size, but low density, indicated that the NSI in chicken serum resembled lipoprotein.

SUMMARY

(1) The non-specific inhibitors (NSI) in normal chicken sera were active against all the tested group A and group B arboviruses, but the group B arboviruses were more sensitive than group A arboviruses.

(2) The titres of the NSI were distributed nearly uniformly among chickens from seven different age groups to group A arboviruses. In contrast, the NSI titres to group A arboviruses were found to increase with age.

(3) No significant difference could be demonstrated between acetone-ether extraction and kaolin adsorption for removal of the NSI in normal chicken sera.

(4) After heating, the NSI titres in chicken sera were increased for both group A and group B arboviruses.

(5) After heating the sera at 80°C and 100°C,

kaolin adsorption was less efficient for removing the NSI than it was in unheated serum. Acetone-ether extraction of the NSI was unimpaired after heating at 80°C but was less efficient after heating at 100°C.

(6) The NSI activity was found mainly in the first peak (IgM) and diffused to a part of second peak (IgG) by fractionation of chicken serum by gel filtration through Sephadex G200. After zonal centrifugation of chicken serum in a linear ten to 40 percent sucrose gradient all of the NSI activities were found on the top of the centrifugal tubes. These properties of large molecular size and low density indicated that the NSI in chicken serum were probably lipoproteins.

(7) The natural agglutinins for goose erythrocytes in chicken sera were partially destroyed by acetone-ether extraction but not by kaolin adsorption, and were efficiently adsorbed with ten percent goose erythrocytes. No difference of the NA titre was demonstrated with diluents of different pH.

(8) The NA in chicken serum was found to possess the properties of IgM by gel filtration through Sephadex G200 and zonal centrifugation in linear ten to 40 percent sucrose gradient.

TABLE 1. Activity of non-specific inhibitor in chicken serum to different arboviruses

Group	Antigen	No. of serum										T
		≦10*	20	40	80	160	320	640	1280	2560	5120	
A	Sindbis	2	16	11	10	5	2	3	1			50
	Getah	1	21	23	3	2						50
	R.R.**	4	11	23	12							50
B	MVE		1	14	16	10	8		1			50
	Kokobera			10	13	14	5	5		1	2	
	Kunjin		2	2	3	16	14	8	5			50
	Edge Hill			1	6	17	6	10	8	1	1	50
	Stratford		2	4	7	14	12	8	2	1		50

* Titre of non-specific inhibitor (Reciprocal of serum dilution)
** Ross River
T: Total tested

TABLE 2. Influence of chicken age on non-specific inhibiting action to group A arboviruses in chicken sera

Antigen	Age of chicken	No. of serum							Total tested
		≦10*	20	40	80	160	320	640	
Getah	5w		6						6
	7		5	1					6
	10		2	4					6
	12		2	4					6
	16		1	4					5
	24		1	5					6
	28		1	3		1			5
	5w	2	4						6
	7		5	1					6
	10		5	1					6

Sindbis	12	2	3	1		6
	16		1	2	2	5
	24		1	4	1	6
	28		2	2	1	5
Ross River	5w	2	4			6
	7	2	4			6
	10		6			6
	12		3	3		6
	16		1	4		5
	24		3	3		6

* Titre of non-specific inhibitor (Reciprocal of serum dilution)
W : Week

TABLE 3. Influence of chicken age on non-specific inhibiting action to group B arboviruses in chicken sera

Antigen	Age of chicken	No. of serum								Total tested
		≦10*	20	40	80	160	320	640	1280	
MVE	5w		1	5						6
	7		1	5						6
	10			3	3					6
	12				3	3				6
	16				2	2	1			5
	24				2	2	2			6
	28				2	2	1			5
Kunjin	5w				1	5				6
	7				1	4	1			6
	10					1	4	1		6
	12					1	4	1		6
	16					1	1	2	1	5
	24					1	1	2	2	6
	28					1		2	2	5
Edge Hill	5w				3	3				6
	7				1	5				6
	10					3	3			6
	12					2	1	2	1	6
	16					1		2	2	5
	24					1		4	1	6
	28					1			4	5

* Titre of non-specific inhibitor. (Reciprocal of serum dilution)

TABLE 4. Effect of heat on non-specific inhibitors of chicken serum to Sindbis virus

Treatment	No. of serum										Total tested
	<10◎	10	20	40	80	160	320	640	1280	2560	
None	2		6	19	16	4	2	1			50
A-E	45		2	1	2						50
Kaolin	42		4	2	1	1					50
60-10+	1	3	1	9	14	11	7	3	1		50
60-30		2	2	9	16	12	6	2	1		50
80-10			2	9	16	9	9	4	1		50
80-30			1	4	17	17	8	2	1		50
100-10			3	3	12	18	8	5	1		50
100-30				6	9	21	8	5	1		50
K60-10++	43	5	1		1						50
K60-30	40	6	4								50
K80-10	16	10	13	7	2	2					50
K80-30	15	9	12	8	3	3					50
K100-10	16	10	8	10	4		2				50
K100-30	17	3	4	17	4	4	1				50
A60-10+++	50										50
A60-30	49	1									50
A60-10	47	3									50
A80-30	47	2	1								50
A100-10	41	6	3								50
A100-30	40	2	3	4	1						50

◎ : Titre of non-specific inhibitor (Reciprocal of serum dilution)

A-E : Extracted with Acetone-ether

+ : Heated at 60°C for 10 minutes

++ : Treated with kaolin after heating at 60°C for 10 minutes

+++ : Extracted with Acetone-ether after heating at 60°C for 10m minutes

TABLE 5. Effect of heat on non-specific inhibitor in chicken serum to MVE virus

Treated with	No. of serum										Total tested
	<10◎	10	20	40	80	160	320	640	1280	2560	
None	1		1	2	10	14	11	8	2	1	50
A-E	41		5	1	3						50
Kaolin	40		5	4	1						50
60-10+	1	2		2	7	17	12	6	3		50
60-30		2	2	3	4	16	13	6	3	1	50
80-10		2		3	9	9	12	10	3	2	50
20-30		2		4	4	7	16	12	3	2	50

100-10		2	1	4	7	10	11	10	3	2	50
100-30		2		2	7	15	12	9	2	1	50
K60-10++	42	5	1	1		1					50
K60-30	40	4	4	2							50
K80-10	11	8	11	7	6	2	5				50
K80-30	13	8	5	9	5	7	3				50
K100-10	7	6	10	9	5	6	7				50
K100-30	11	5	6	3	8	9	8				50
A60-10+++	45	5									50
A60-30	42	4	2	1		1					50
A80-10	39	11									50
A80-30	39	5	3	3							50
A100-10	36	5	2		1	5	1				50
A100-30	30	9	2	3	1	3	2				50

◎ : Titre of non-specific inhibitor (Reciprocal of serum dilution)

A-E : Extracted with Acetone-ether

+ : Heated at 60°C for 10 minutes

++ : Treated with kaolin after heating at 60°C for 10 minutes

+++ : Extracted with Acetone-ether after heating at 60°C for 10 minutes

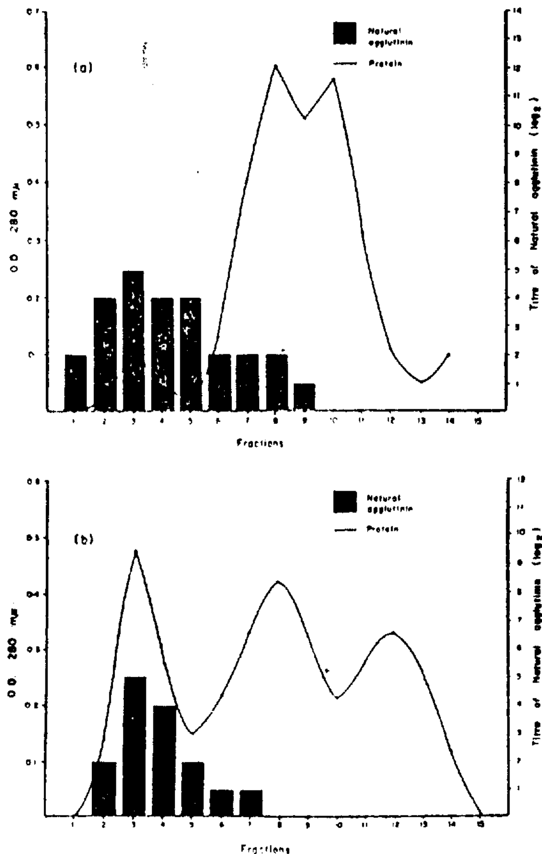

Figure 1.

(a) Five normal chicken sera were pooled and 0.4ml of the pooled serum was subjected to zone centrifugation. Centrifugation was performed at 35,000rpm for 18 hours in swinging buckets rotor SW39. Titre of natural agglutinin is reciprocal dilution of the fractions. Protein were measured with UV-Spectrophotometer with wave length at 280mμ.

(b) Five normal chicken sera were pooled and one ml of the pooled serum was filtered through a Sephadex G200 column. Titre of natural agglutinins is reciprocal dilution of the fractions. Protein was measured with UV-Spectrophotometer with wave length at 280mμ.

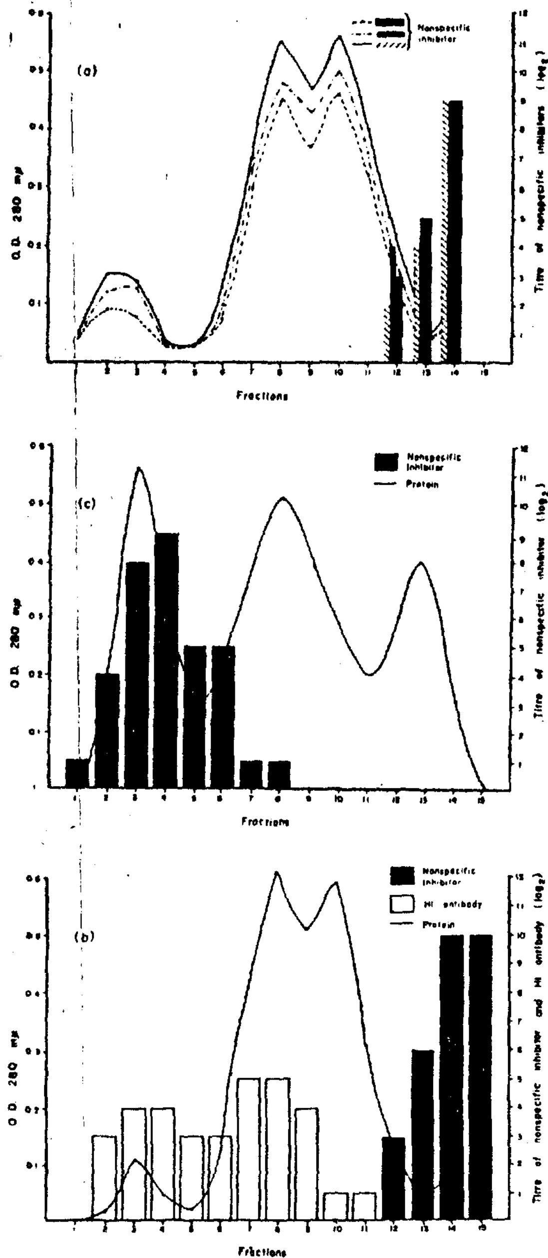

Figure 2.

(a) 0.4ml each of three normal chicken sera and

(b) one chicken immune serum were subjected to zone centrifugation in a linear ten to 40 percent sucrose gradient. Centrifugation was performed at 35,000 rpm for 18 hours in swinging buckets rotor SW39. Titres of non-specific inhibitor and HI antibody are reciprocal dilution of the fractions. Protein was measured with UV-Spectrophotometer with wave length at 280mμ.

(c) Five normal chicken sera were pooled and one ml of the pooled serum was filtered through a Sephadex G200 column. Titre of non-specific, inhibitors is reciprocal dilution of the fractions. Protein was measured with UV-Spectrophotometer with wave length at 280mμ.

Table 6. Effect of pH on natural agglutinin of chicken serum erythrocytes

pH	No. of sera							Total tested
	10*	20	40	80	160	320	640	
6.5	10	18	10	7	3	2		50
6.7	10	16	14	6	2	1	1	50
6.9	12	13	10	10	3	2		50
7.1	8	16	13	10	2	1		50

* Titre of agglutinin (Reciprocal of serum dilution)

Table 7. Effect of acetone-ether and kaolin treatment on natural agglutinins of erythrocytes in chicken serum

Treatment	No. of sera						Total tested
	10*	10	20	40	80	160	
None	4	12	22	8	19	5	70
Kaolin	4	17	17	12	14	6	70
A-E**	18	27	15	0	0	0	70

* Titre of agglutinins (Reciprocal of serum dilution)

** Acetone-ether extraction

Adjusting diluent of pH 6.7 was used for diluent of goose erythrocytes

Table 8. Heat stability of natural agglutinins of chicken serum to goose erythrocytes and HI antibody to MVE virus

Temp. (°C)	Time (min.)	serum Titre*		
		Natural agglutinin	HI antibody 1:2**	1:4
0	0	80	640	320
56	30	80	640	320
60	30	80	640	320
70	10	0	640	320
	20	0	160	80
	30	0	40	<20
80	10	0	—	—
	20	0	—	—
	30	0	—	—
90	10	0	—	—
	20	0	—	—
	30	0	—	—

* Reciprocal of serum dilution

— Not tested

** Reciprocal dilution of test serum

Table 9. Removal of natural agglutinins in chicken serum by goose erythrocytes

Serum No.	Goose cells		Titre of natural agglutinin					
	%	time	10	20	40	80	160	320
1	0	0	+	+	+	+	–	–
	10	10	–	–	–	–	–	–
		20	–	–	–	–	–	–
	20	10	–	–	–	–	–	–
		20	–	–	–	–	–	–
2	0	0	+	+	+	+	+	
	10	10	–	–	–	–	–	–
		20	–	–	–	–	–	–
	20	10	–	–	–	–	–	–
		20	–	–	–	–	–	–
3	0	0	+	+	+	+	+	–
	10	10	–	–	–	–	–	–
		20	–	–	–	–	–	–
	20	10	–	–	–	–	–	–
		20	–	–	–	–	–	–

+ Natural agglutinins present

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鷄血淸에 들어 있는 腦炎바이러스에 대한 비특이물질 및 거위 血球에 대한 응집소의 性狀에 관한 硏究

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鄭 榮 錫

抄 錄

이 實驗은 鷄血淸에 들어 있는 腦炎바이러스에 대한 非特異抑制物質(NSI)의 性狀과 이의 Acetone-ether와 Kaolin 處理에 의한 NSI의 除去効力등을 Gel filtration 및 Zonal Centrifugation technique를 이용하여 시험 하였다. 그 結果

(1) 鷄血淸에 들어 있는 비특이 억제물질은 Group A arbovirus 보다 Group B arbovirus에 대하여 더 예민하였다.

(2) 年齡에 의한 NSI의 力價차이는 없었다.

(3) NSI를 除去하기 위한 Acetone-ether 및 Kaolin 치리는 그 효력면에서 차이가 없었다.

(4) 鷄血淸을 일정한 溫度로 加熱하면 NSI의 力價가 上昇 하였다.

(5) NSI의 成分은 Gel filtration에서 주로 첫 Peak (IgM)에 있었고, Zonal Centrifugation에서는 원심관의 上層에 있는 것으로 보아, 이것의 分子量은 크고 Density는 낮은 脂質蛋白으로 생각된다.

(6) 거위 적혈구에 대한 응집소(NA)는 acetone-ether 처리에 의하여 部分的으로 파괴 되었으나 Kaolin 처리에서는 그렇지 못하였고, 10% 거위혈구액으로써 완전 제거할 수 있었다.

(7) NA는 Gel filtration 및 Zonal Centrifugation에서 첫 peak (IgM)에 나타났다.