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The Conglutination Phenomenon

XIV. THE RESISTANCE ENHANCING EFFECT OF CONGLUTININ AND IMMUNO-CONGLUTININ IN EXPERIMENTAL BACTERIAL INFECTIONS

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Summary. The effect of conglutinin and immuno-conglutinin preparations on the resistance of animals has been studied in seven bacterial infections. In six infections —*Salmonella typhimurium*, *Pasteurella septica*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*—higher survival rates were demonstrated in mice previously injected with the conglutinin preparations.

Infection with avirulent strains or non-pathogenic organisms in numbers large enough to cause death led to severe toxaemia against which the injection of conglutinin preparations failed to protect. Although the conglutinin preparations failed to protect against avirulent or unadapted strains, they did protect against virulent or adapted strains of the organisms.

Experiments undertaken to define the protective factor in the serum preparations indicate that the protective factor probably is the same as that which is responsible for the conglutinating activity, conglutinin.

INTRODUCTION

Marks and Coombs (1957) demonstrated that higher-than-normal levels of conglutinating activity were present in the sera of human patients suffering from infective diseases and other diseases where an antigenic challenge is involved. Similarly, Ingram, Barber, McLean, Soltys and Coombs (1959) have shown a raised conglutinating activity in experimental infections in animals. The immuno-conglutinin and specific immune antibody begin to rise and reach their peaks at the same time. In acute infections, after reaching the peak titre the level of conglutinating activity drops more rapidly than the level of specific antibody. In chronic infections the increased level of conglutinating activity is maintained for a considerable time.

Ingram (1959) has presented evidence that if conglutinin is present in the serum of an animal the level of conglutinating activity decreases markedly immediately after an antigenic challenge in the form of a bacterial infection. Ingram also reported that the prior administration of a conglutinin or an immuno-conglutinin preparation increases the bactericidal activity of the body defences.

It was hypothesized that immuno-conglutinin may be one of the serum factors which aid the animal host in its struggle against an invading organism. The present paper reports the results of some experiments undertaken to test this hypothesis. Mice with demonstrable conglutinating activity in their serum were more resistant than control mice to the fatal effects of several experimental bacterial infections.

MATERIALS AND METHODS

The normal young-adult mice of 24 to 30 gm. used in these experiments were derived from an inbred strain (FF), but inbreeding had not been practised for some time in this

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colony. Ingram *et al.* (1959) have shown that these mice normally have no, or very low, conglutinating activity in their serum.

The conglutinin preparation was the euglobulin precipitate of normal bovine serum redissolved in saline (Ingram, 1959) and had a titre of 1 in 640. Rabbit serum containing immuno-conglutinin was produced by the intravenous injection of kaolin treated with active horse serum as described by Coombs and Coombs (1953). The pool of several such sera had a titre of 1 in 8000 and this serum pool was used without purification as a source of immuno-conglutinin. Both serum preparations were heated at 56° C. for 30 minutes before use and both transferred conglutinating activity to the serum when injected subcutaneously into the mouse (Ingram, 1959).

TABLE 1

SOURCE AND IDENTIFICATION OF BACTERIAL CULTURES

Organism	*Source*	*Identity*
Salm. typhimurium	Dept. of Path., Camb. Univ.	—
Esch. coli 0111 B4	National Collection of Type Cultures	8958
Past. septica (mouse)	" " " " "	1140
Kleb. pneumoniae	" " " " "	9735
Kleb. pneumoniae	" " " " "	6869
List. monocytogenes	Dept. of Animal Path., Camb. Univ.	—
Strep. pneumoniae type 1	National Collection of Type Cultures	7465
Strep. pyogenes	Lister Institute, London	CN771

The source and identification of the bacteria which were tested are shown in Table 1. The bacteria were grown at 37° C. for 18 hours on blood agar and, except where specifically noted, were harvested into cold saline. An estimate of the number of organisms was obtained by plate counts of ten-fold serial dilutions of a sample of this suspension. The bacterial suspension was stored overnight at 4° C. until the number of organisms per ml. was calculated and was then diluted the appropriate amount for challenge. At the time of challenge a second series of plate counts were made to check on the number and viability of the organism.

The ten-day LD_{50} was calculated by the method of Irwin and Cheeseman (1939) from five or more groups of five mice challenged intraperitoneally with ten-fold dilutions of a standardized bacterial suspension.

As a critical test of increased resistance, ten treated mice were injected subcutaneously with one of the serum preparations and were challenged intraperitoneally 24 hours later. As controls for the conglutinin preparation ten mice were injected with an equal volume of sterile saline, and as controls for the immuno-conglutinin preparation, ten mice were injected with an equal amount of normal rabbit serum. Necropsies were performed on all dead mice and bacterial cultures made from the heart-blood, liver and spleen. The specific infecting organism was isolated from all dead animals reported in these experiments.

All previous reports on the absorption of immuno-conglutinin have involved the use of complement-treated bacterial cells. It was undesirable to use bacteria for the absorption of serum because of the marked effect which bacterial products have on non-specific immunity. Therefore for section II (d) a technique of absorbing immuno-conglutinin using 'alexinated' red blood cells as the absorbing antigen was developed.

Absorption Procedure:

Sensitized cells were prepared by mixing equal volumes of 8 per cent sheep erythrocytes and inactivated normal bovine serum and incubating at 37° C. for 15 minutes. The cells were then washed once and resuspended in saline to make a 10 per cent suspension of sensitized cells.

The alexinated cells were prepared by mixing 10 ml. of 10 per cent sensitized cells, 7·5 ml. of active horse serum and 7·5 ml. of inactivated horse serum and incubating at room temperature for 30 minutes. Then the cells were sedimented by mild centrifugation, washed twice in saline and finally centrifuged to give 1 ml. of packed alexinated cells.

The 'control' cells were prepared in the same manner as the alexinated cells except that the 7·5 ml. of active serum was replaced by an equal volume of inactivated horse serum.

The serum containing immuno-conglutinin was diluted 1 in 4 in saline and 4 ml. of diluted serum was absorbed ten times with 1 ml. volumes of freshly prepared packed cells. Absorptions with alexinated and 'control' cells were conducted in parallel. Each absorption was for 15 minutes with five absorptions at room temperature and five at 37° C.

TABLE 2

ENHANCED RESISTANCE OF MICE INJECTED WITH CONGLUTININ OR IMMUNO-CONGLUTININ PREPARATION TO *Salmonella typhimurium* INFECTION

Preparation tested	*Number of* LD_{50}	*Survivors at 10 days*	
		Treated	*Control*
Conglutinin	20	8/10*	4/10
	20	5/10	1/10
	20	6/10	1/10
Total		19/30	6/30
Immuno-conglutinin	20	8/10	2/10
	20	7/10	0/10
	20	10/10	0/10
	20	8/10	3/10
	20	6/9	0/9
Total		39/49	5/49

The conglutinin or immuno-conglutinin preparation was injected subcutaneously 24 hours before challenge.
* No. of survivors/No. tested.

RESULTS

1. THE EFFECT OF THE CONGLUTININ AND IMMUNO-CONGLUTININ PREPARATIONS ON RESISTANCE TO BACTERIAL INFECTIONS

(*a*) *Salmonella typhimurium Infection*

The *Salm. typhimurium* strain was from a stock culture and no attempt was made to increase its virulence. The LD_{50} was 1×10^4 and had a large standard error. Therefore, 20 LD_{50} was chosen as the standard challenge. Tests with four-fold dilutions of the

immuno-conglutinin preparation showed that 0·5 ml. of a 1 in 4 dilution gave the best protection and thus 0·125 ml. of the preparations was used as the standard subcutaneous injection into test mice.

The data from a number of experiments using the conglutinin or immuno-conglutinin preparation are presented in Table 2 and show that both preparations significantly increase the resistance of mice to this infection. *Salm. typhimurium* infection was utilized throughout many of the experiments reported in this series of papers, and both the conglutinin and immuno-conglutinin preparations have consistently shown protective properties.

(*b*) *Escherichia coli Infection*

The *Esch. coli* 0111B4 strain had an LD_{50} of 2×10^8 with a relatively small standard error. This non-pathogenic organism was tested using 2 and 5 LD_{50} challenges, but, as shown in Table 3, no protective effect was demonstrated. The injection of the large number of organisms necessary to cause death produced a marked toxaemia which became evident in all animals within an hour after infection. Most deaths occurred within 36 hours and mice which survived for two days usually recovered. This pattern of infection and deaths was in marked contrast to the *Salm. typhimurium* infection in which deaths were first recorded from the third or fourth day and continued throughout the test period.

TABLE 3

RESISTANCE OF MICE INJECTED WITH IMMUNO-CONGLUTININ SERUM TO INFECTION WITH GRAM NEGATIVE ORGANISMS

Infecting organism	*Number of* LD_{50}	*Survivors at 10 days*	
		Treated	*Control*
Esch. coli 0111 B4	5	0/10	3/10
	2	1/10	3/10
Past. septica	10	0/10	0/10
	5	3/10	1/10
Kleb. pneumoniae 9735	5	0/10	2/10
	2	5/10	7/10
Kleb. pneumoniae 6869	4	10/10	2/10
	4	8/10	0/10

All mice injected subcutaneously with immuno-conglutinin serum or normal serum 24 hours before challenge.

(*c*) *Pasteurella septica Infection*

The strain of *Past. septica* was of mouse origin (N.C.T.C. information), but the LD_{50} was $1{\cdot}2 \times 10^8$. (This organism did not survive well at low temperature and much better survival was obtained by harvesting and storing the organism at room temperature during the standardization period.) The data from two tests with this organism are shown in Table 3. No significant protection was given by the immuno-conglutinin preparation.

By ten serial passages of this organism through mice, the LD_{50} was reduced to 4×10^6. This adapted strain was tested in several experiments and in this infection two periods of death were evident. The first occurred during the first five days after infection. During

this time all mice showed symptoms of a marked toxaemia: apathy, ruffled coat and anorexia. Animals which survived this period improved clinically and were normal in appearance by the fifth or sixth day. The second period of deaths occurred after the fifth day and continued up to 30 days after infection, when all survivors were killed. During this second period individual animals would have a regression to the early symptoms and the mouse would die.

TABLE 4

RESISTANCE OF MICE INJECTED WITH IMMUNO-CONGLUTININ SERUM TO *Pasteurella septica* INFECTION

Preparation injected	*Survivors*		
	5 *days*	10 *days*	30 *days*
Immuno-conglutinin serum	17/60	16/60	14/60
Normal serum	24/60	15/60	5/60

All mice were challenged intraperitoneally with 5 LD_{50} of the organism 24 hours after subcutaneous injection of the rabbit serum.

These experiments were conducted after the virulence of the organism had been enhanced by ten serial passages through mice.

The effect of the immuno-conglutinin serum was interesting in the infection with this adapted strain because no protection was given against the first period of deaths, but the immuno-conglutinin treated animals survived the second period of deaths much better than the controls. Table 4 shows the combined results of several experiments for the time intervals of 5, 10 and 30 days post-infection and represents the findings in all experiments. The immuno-conglutinin serum did not protect mice against the early 'toxic' death, for the treated animals always had a higher mortality during this period than the controls. However, the immuno-conglutinin treated animals which survived the first 5 days had a much lower mortality than the controls during the second period of deaths. It appears that the immuno-conglutinin serum had modified the course of the infection during the early stages and had enabled the mice to resist the establishment of the long-lasting infection which caused the later deaths.

(*d*) *Klebsiella pneumoniae Infection*

Two strains of *Kleb. pneumoniae* were tested and both were given ten serial passages through mice before testing. Strain 9735 was relatively avirulent and had an LD_{50} of 3×10^7. The data from two tests with this strain are shown in Table 3, which shows that the immuno-conglutinin-treated animals did not have an increased resistance to this infection. The injection of strain 9735 in numbers large enough to cause death resulted in severe toxic symptoms which became apparent within a few hours after infection. All animals which died showed an increasing depression and death occurred within 2 days. The immuno-conglutinin treated animals had a slightly higher mortality than the controls in this infection.

Strain 6869 was virulent for mice and had an LD_{50} of 8×10^4. The data from two tests with this strain, also presented in Table 3, demonstrate a high degree of resistance

in the treated mice as compared with the controls. No toxic symptoms were apparent following infection with strain 6869, and this is probably a reflection of the smaller number of organisms injected. Mice infected with this strain remained normal in appearance until the third day, when some animals became sick and most deaths occurred from the fourth to sixth day. The immuno-conglutinin treated mice had a much lower mortality than the controls in this infection.

The two strains showed different types of infection, different times of death and a difference in the protective capacity of immuno-conglutinin serum. The results of the experiments with this species may aid in the interpretation of the data from infection with other Gram-negative organisms. It would seem that the conglutinin preparations are protective against what may be termed true infectious disease, but are not protective against the toxaemia caused by the injection of large numbers of organisms.

(*e*) *Listeria monocytogenes Infection*

The strain of *List. monocytogenes* was from a stock culture and was tested both before and after adaptation by twenty serial passages through mice. The LD_{50} of the unadapted organism was 7×10^7. As shown in Table 5, several tests with this organism failed to demonstrate any protective effects of either the conglutinin or immuno-conglutinin preparations.

TABLE 5

RESISTANCE OF MICE INJECTED WITH THE CONGLUTININ OR IMMUNO-CONGLUTININ PREPARATION TO *Listeria monocytogenes* INFECTION

Infecting organism	*Preparation tested*	*Number of LD_{50}*	*Survivors at 10 days*	
			Treated	*Control*
List. monocytogenes (unadapted)	Conglutinin	5	0/10	1/10
		2	1/10	0/10
		1	7/10	10/10
	Immuno-conglutinin	5	0/10	0/10
		2	0/10	2/10
		2	0/10	1/10
List. monocytogenes (adapted)	Immuno-conglutinin	5	5/10	0/10
		5	4/10	1/10
		2	7/10	4/10

All mice were injected subcutaneously with the serum preparations 24 hours before challenge.

After passage the LD_{50} was 4×10^5. The data from three tests with the adapted strain, presented in Table 5, show that the immuno-conglutinin serum did increase the resistance of mice to this more virulent strain.

(*f*) *Streptococcus pneumoniae Infection*

The *Strep. pneumoniae* type I was tested as it was received from N.C.T.C. and had an LD_{50} of $7{\cdot}5\times10^3$. Then the virulence was enhanced by ten serial mouse passages and the adapted strain which had an LD_{50} of $2{\cdot}4\times10^3$ was retested. The data from these tests are

presented in Table 6 and demonstrate that although immuno-conglutinin serum did not protect against the unadapted organism it gave good protection against the adapted strain.

TABLE 6

RESISTANCE OF MICE INJECTED WITH IMMUNO-CONGLUTININ SERUM TO INFECTION WITH GRAM POSITIVE ORGANISMS

Infecting organism	*Number of* LD_{50}	*Survivors at 10 days*	
		Treated	*Control*
Strep. pneumoniae type I (unadapted)	3	2/10	4/10
	1	7/10	8/10
Strep. pneumoniae type I (adapted)	2	9/10	2/10
	2	9/10	1/10
Strep. pyogenes	5	5/10	3/10
	4	8/10	4/10

All mice were injected subcutaneously with immuno-conglutinin serum or normal serum 24 hours before challenge.

(*g*) *Streptococcus pyogenes Infection*

The *Strep. pyogenes* which was given ten serial mouse passages before being tested had an LD_{50} of $3{\cdot}4 \times 10^2$. The data from two tests with this organism are presented in Table 6 and show that the immuno-conglutinin preparation increased the resistance of mice to this virulent organism.

II. EXPERIMENTS TO DEFINE THE RESISTANCE ENHANCING FACTOR

The experiments which have been described compared the resistance of mice injected with either bovine euglobulin or rabbit serum containing immuno-conglutinin to control mice injected with sterile saline or normal rabbit serum, respectively. The injection of the conglutinin or immuno-conglutinin preparations increased the non-specific immunity of mice. Many serum components other than conglutinin or immuno-conglutinin were present in both preparations and the specific involvement of conglutinin or immuno-conglutinin in this augmented resistance could not be said to have been demonstrated. The experiments reported in this section were attempts to establish whether conglutinin or immuno-conglutinin were actual agents in this non-specific immunity to bacterial infections.

Salm. typhimurium infection was utilized as the standard challenge throughout this series of experiments.

(*a*) *The Period of Increased Resistance*

The subcutaneous injection of rabbit serum containing immuno-conglutinin transfers conglutinating activity to the serum of a recipient mouse which reaches a peak within 1 or 2 days, then slowly decreases until at 12 to 14 days no significant conglutinating activity can be demonstrated (Ingram, 1959). Experiments were performed to find

whether the increased resistance paralleled the conglutinating activity of the serum over this period.

Groups of 10 mice were injected with immuno-conglutinin serum at intervals so that the time of injection ranged from 14 days before, until 2 days after, challenge. One control group was injected with normal rabbit serum 2 days before infection. All mice were challenged at one time with the same suspension of *Salm. typhimurium*. This test has been repeated with similar results both times.

The data from one experiment are presented in Table 7 and show that the increased resistance had a course similar to that of the increased conglutinating activity of the serum. In this test the mice injected with immuno-conglutinin serum 2 days before challenge showed the greatest resistance, while control mice injected at this time all died. Mice injected with immuno-conglutinin serum at the time of infection were partially protected, but those injected 2 days after infection were not.

TABLE 7

PERIOD OF INCREASED RESISTANCE OF MICE INJECTED WITH IMMUNO-CONGLUTININ SERUM

Preparation injected	*Day of injection*	*Survivors*	
		10 *days*	50 *days*
Immuno-conglutinin serum	—14	3/10	1/10
" " "	— 7	8/10	4/10
" " "	— 2	9/10	9/10
Normal serum	— 2	0/10	0/10
Immuno-conglutinin serum	0	7/10	7/10
" " "	+2	0/10	0/10

All mice challenged with $20LD_{50}$ of *Salm. typhimurium* on day 0.

— indicates number of days before challenge.

+ indicates number of days after challenge.

To determine whether the protection by immuno-conglutinin serum enabled the mice to survive for a prolonged period the survivors were maintained for 50 days. The survival data shown on Table 7 show that the protection was long-lasting.

(b) The Resistance Enhancing Effect of Bovine and Rabbit Euglobulins

The conglutinin of bovine serum is precipitated in the euglobulin fraction, while that of the rabbit serum remains in the supernatant pseudoglobulin. Experiments were conducted comparing the resistance enhancing effect of bovine and rabbit euglobulins.

The euglobulins were precipitated from fresh normal bovine and rabbit sera by dialysis against distilled water for 48 hours at 4° C. The precipitates were dissolved in saline to the original serum volume and standard amounts of these preparations were injected subcutaneously into mice. The animals were challenged one day later with the results in Table 8.

Mice which were injected with rabbit euglobulin had a mortality equal to uninjected control animals, but mice injected with bovine euglobulin had a significantly lower mortality.

TABLE 8

RESISTANCE ENHANCING EFFECT OF BOVINE AND RABBIT EUGLOBULIN PREPARATIONS

Preparation injected	*Survivors at 10 days*	
	Expt. 1	*Expt. 2*
Bovine euglobulin preparation	5/10	9/10
Rabbit euglobulin preparation	1/10	0/10
Controls (not injected)	—	0/10

All mice were injected with the euglobulin preparations 24 hours before challenge with $20LD_{50}$ of *Salm. typhimurium*.

(*c*) *Increased Resistance in Mice following Active Stimulation of Immuno-Conglutinin*

The protective effect of rabbit serum containing immuno-conglutinin has been repeatedly demonstrated. This experiment was designed to find if mice stimulated to produce immuno-conglutinin had an increased resistance to infection.

One group of ten mice was stimulated to produce immuno-conglutinin. Each mouse received three intravenous injections of 0·1 ml. of kaolin treated with active horse serum (Coombs and Coombs, 1953) at 2-day intervals and was challenged 6 days after the last injection. Other mice treated similarly had a conglutinating activity in their serum to a titre of 1 in 40 to 1 in 80.

Another group of ten mice was injected with the rabbit serum containing immuno-conglutinin 24 hours before challenge.

A third group of ten mice served as untreated controls.

All mice were challenged intraperitoneally with $20LD_{50}$ of *Salm. typhimurium* at the same time. The resulting data, presented in Table 9, show that both groups of mice which had conglutinating activity in their serum had significantly greater resistance than the controls.

TABLE 9

THE INCREASED RESISTANCE OF MICE ACTIVELY STIMULATED TO PRODUCE IMMUNO-CONGLUTININ

Preparation injected	*Survivors at 10 days*	
	Expt. 1	*Expt. 2*
Kaolin treated with active equine serum*	7/10	8/10
Immuno-conglutinin serum†	9/10	9/10
Normal controls (not injected)	3/10	2/10

* Mice injected intravenously 10, 8 and 6 days before challenge.
† Mice injected subcutaneously 24 hours before challenge.
All mice were challenged with $20LD_{50}$ of *Salm. typhimurium*.

(*d*) *Lack of Protective Property of the Serum after the Specific Absorption of Immuno-Conglutinin*

The serum containing immuno-conglutinin was produced in rabbits by the injection of kaolin treated with active horse serum. To minimize the variation of serum components

other than immuno-conglutinin the control serum was obtained from the same rabbits before the injection of kaolin. However, factors other than immuno-conglutinin may have been present in the serum which could account for the protective property. Therefore, the serum from which the immuno-conglutinin had been absorbed was tested for its resistance enhancing effect.

Absorption with alexinated cells as described under *Materials and Methods* removed the conglutinating activity of the serum, while absorption with control cells did not significantly reduce this activity.

TABLE 10

ATTEMPTED REMOVAL OF THE PROTECTIVE PROPERTY OF THE IMMUNO-CONGLUTININ SERUM BY ABSORPTION OF ITS IMMUNO-CONGLUTININ

Preparation injected	*Survivors at 10 days*			
	Expt. 1	*Expt.* 2	*Expt.* 3	*Expt.* 4
Immuno-conglutinin serum absorbed with alexinated cells	8/10	4/10	2/10	5/10
Immuno-conglutinin serum absorbed with 'control' cells	10/10	8/10	—	—
Immuno-conglutinin serum (unabsorbed)	—	—	8/10	8/10
Normal serum	5/10	2/10	1/10	3/10

Mice were injected subcutaneously with the serum preparations 24 hours before challenge with 20LD_{50} of *Salm. typhimurium*.
— indicates that this preparation was not tested.

The data from several experiments using these preparations are presented in Table 10. Absorption of the serum with control cells did not remove the conglutinating activity or the protective power of the serum. Absorption with alexinated cells, on the other hand, removed the conglutinating activity and at least the greater part of the protective potential of the serum.

DISCUSSION

The increased resistance to several antigenically distinct organisms indicates that the protective factor is not related to the antigenic characteristics of the infecting organism. Coombs (1947) reported that the euglobulin from bovine serum contained the conglutinin, but the supernatant pseudoglobulin contained the antibodies to sheep cells and *Brucella*. The bovine euglobulin preparation used in these experiments did not contain agglutinins for *Salm. typhimurium*. Both the normal rabbit serum and the rabbit serum containing immuno-conglutinin agglutinated *Salm. typhimurium* when tested undiluted but failed to cause agglutination at a dilution of 1 in 4. Specific immune antibodies were not present in the serum preparations used in these experiments.

The conglutinin preparations failed to protect mice against a non-pathogenic species (*Esch. coli*) or avirulent strains of pathogenic organisms. Infection with such strains led to a rapidly developing toxaemia in which the mice treated with the conglutinin preparations had a higher mortality than control mice. Infection with *Past. septica* gave rise to

two periods of death. The first occurred during the first 2 or 3 days after infection and the second from day 5 to day 30. The immuno-conglutinin serum made the mice slightly more susceptible during the first period of deaths but more resistant during the second period. Two strains of *Kleb. pneumoniae* gave somewhat similar results. The avirulent strain caused death during days 1 and 2 and no protection was afforded by the immuno-conglutinin serum. The virulent strain caused deaths from days 4 to 6 and immuno-conglutinin serum protected against this infection. The data from the infections with these Gram-negative organisms and with adapted and unadapted strains of *List. monocytogenes* and *Strep. pneumoniae* indicate that the conglutinin preparations give significant protection against true infectious diseases but fail to protect against the toxaemia caused by the injection of a large number of bacteria.

Experiments (not reported here) with these same bacteria showed that the enchanced resistance due to the conglutinin preparations, like that of specific antibody, can be overcome by large challenge doses of organisms.

In this series of tests the increased non-specific immunity was associated with the conglutinating activity of the serum of the animal. Rabbit euglobulin which does not contain conglutinin failed to protect mice in the same manner as bovine euglobulin. The rise and fall of the increased resistance to *Salm. typhimurium* and of the conglutinating activity of the serum of mice injected with immuno-conglutinin serum followed similar courses both in time and in degree.

Animals stimulated to produce immuno-conglutinin showed an increased resistance to infection. In other experiments two rabbits stimulated to produce immuno-conglutinin by the intraperitoneal injection of killed *List. monocytogenes* were more resistant than normal rabbits to oral infection with *Salm. typhimurium* (Ingram, 1959). The stimulus used in mice was a suspension of complement-treated kaolin and thus the increased resistance is not associated with any similar antigenic characteristic of the material injected for stimulating immuno-conglutinin and the bacteria used as a challenge.

The specific absorption of immuno-conglutinin from the protective serum removed at least the greater part of its protective property, thus indicating that immuno-conglutinin is a protective factor. It is believed that this experiment presents strong evidence that immuno-conglutinin is involved in the increased resistance illustrated in this series of experiments. Another crucial experiment to test the effect of these substances on non-specific immunity would be to test pure conglutinin or immuno-conglutinin, but, to date, neither of these substances has been purified.

The activity of conglutinin, or immuno-conglutinin, is believed to be specific for adsorbed complement and unrelated to the antigenic characteristics of the infecting organism. The *in vivo* adsorption of complement on an invading organism would make it susceptible to the action of conglutinin or immuno-conglutinin which may thus enhance the normal defence mechanisms of the host. Evidence has been presented that conglutinin and immuno-conglutinin are factors involved in non-specific immunity.

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