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# Some Serological Characteristics of Normal Incomplete Cold Antibody

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**Summary.** The finding that the presence of normal incomplete cold antibody in cord serum is independent of its presence in the corresponding maternal sample suggests that the antibody is produced by the newborn infant. The observation that the antibody has a constant titre in newborn infants aged between a few weeks and 6 months points to the same conclusion.

The normal incomplete cold antibody differs from other blood group antibodies in its thermolability, in being present in the serum of patients with severe hypogammaglobulinaemia and in its interaction with some polysaccharides and bacteria. In many respects the antibody resembles properdin, but markedly differs from properdin in its specificity and other serological characteristics.

## INTRODUCTION

Human red cells left in contact with normal human serum at 0° for 2 hours and then washed with warm saline, in order to elute the cold agglutinins, give a positive reaction when tested with a suitable anti-globulin serum. Dacie (1950) who described this reaction, termed the antibody responsible the 'normal incomplete cold' antibody.

The normal incomplete cold (n.i.c.) antibody is firmly bound to the red cells only in the presence of complement (Dacie, 1950) and cannot be detected directly in the anti-globulin test but only indirectly by the ability to bring about the binding of complement; thus red cells sensitized with n.i.c. antibody are not agglutinated by an anti-$\gamma$-globulin serum but are agglutinated by antisera which react with complement components (Dacie, Crookston and Christenson, 1957). Under appropriate conditions the antibody will lyse normal human red cells (Mollison and Thomas, 1959) and sensitize them to an immuno-conglutinin (I-K) serum (Mollison and Polley, 1960).

It has been shown that the antibody is present in cord serum (Crawford and Mollison, 1956) but that it is not a $\gamma$G-globulin and indeed has properties different from classical blood group antibodies (Adinolfi, Daniels and Mollison, 1963).

This paper reports some further observations on the nature of the n.i.c. antibody and its incidence in the serum of newborn infants, normal adult subjects and patients with hypogammaglobulinaemia.

## MATERIALS AND METHODS

*Human sera*

The frequency of the n.i.c. antibody in a normal population was studied by testing serum from forty-five random donors; the serum, separated as soon as possible from fresh

clotted blood, was stored at −50° for not more than 1 week before being used. In relation to the ABO blood groups the forty-five donors were distributed as follows: $A_1B$: 2; $A_2B$: 1; $A_1$: 12; $A_2$: 5; B: 6; O: 19.

In studying the relation between the titre of the n.i.c. antibody and the ability to secrete A, B and H substances, sera from twelve additional group O donors were tested. The sera were selected in order to obtain two equal groups of 'secretors' and 'non-secretors'. Further sera and samples of saliva were obtained from ten families with twenty parents and forty-one children.

Twenty-three pairs of maternal and cord sera were tested; the cord blood was obtained from the umbilical vein; the maternal samples were taken by venepuncture within 2 days of delivery.

Eleven infants, from 3 to 15 weeks old, were bled by heel prick, at least 1 ml of blood being collected; six of these infants were born prematurely, but were otherwise normal. One of the infants was bled three times within a period of 6 weeks.

Seven normal infants, born at term, were bled by venepuncture three times at intervals of about 5 weeks. These infants were from 7 to 26 weeks old and they were receiving a normal course of vaccination with oral poliomyelitis vaccine (Sabin) and with a combined diptheria, tetanus and pertussis vaccine, following the schedule and the dosage described by Butler, Benson, Urquhart, Goffe, Knight and Pollock (1964) (see section 'Study 1, group B').

The sera from fifteen patients with hypogammaglobulinaemia were kindly supplied by the Medical Research Council Working Party; six of these sera were tested only a few weeks after being collected and had been stored at −50°; the other eight sera had been stored at −20° for many years; estimates of the concentration of γ-globulins were kindly supplied by Dr J. F. Soothill. All sera were tested for anti-A, anti-B and anti-I agglutinins. Some sera were also tested for bacterial antibodies.

*Polysaccharides and simple sugars used in inhibiting the n.i.c. antibody*

Zymosan samples (type A) were obtained from Fleishman Laboratory, U.S.A.; Light & Co., England and I.S.M., Italy. The soluble dextran was produced by Bengers Laboratories Ltd, England (Dextraven; 6 per cent dextran in saline); potato starch was obtained from B.D.H., U.K. and Griffin & George, U.K.; D-glucose, L-fucose were obtained from B.D.H. and Inulin (from Dahlia) from Difco Lab., U.S.A.

*Bacteria*

The samples of bacteria were kindly supplied by Dr A. A. Glynn. *E. coli* C3; *E. coli* A55; *Streptococcus aureus* 80; *Salmonella enteritidis* variety Danysz and *Pneumococcus* type XIV were grown on nutrient agar, killed by heating at 60° for 1 hour and washed three times with distilled water. The bacteria were then weighed (wet weight), re-suspended in buffered saline to give approximately 10 mg of bacteria in 1 ml and were stored at 4°, if not used immediately. Before being used the bacteria were washed again with buffered saline.

*Sera used as a source of complement*

Horse serum was separated within a few hours from freshly drawn blood and absorbed at 0° three or four times with previously washed human red cells. The absorbed serum was partitioned into small aliquots and stored at −50°. Fresh human serum was absorbed three times at 0° for 3 or 4 hours with group O red cells.

*Immunoconglutinin (I-K) serum*

Two sera were prepared in rabbits by giving repeated injections of a killed suspension of *Salmonella pullorum*; the doses were those described by Mollison and Polley (1960), based on Coombs and Coombs (1953).

*Serological tests for detecting the n.i.c. antibody*

Ten volumes of each of a series of doubling dilutions of the antibody-containing serum were incubated at 0° for 2 hours with one volume of a 50 per cent suspension of fresh group O red cells and one volume of complement; the red cells were then washed three times with warm saline, in order to elute the cold agglutinins, and finally tested with a suitable immune serum on an opal tile.

In many experiments fresh horse serum, previously absorbed with human red cells, was used as a source of complement and the uptake of complement was detected by using an (I-K) serum. In a few experiments fresh human serum absorbed three times with group O red cells was used as a source of complement and the antiglobulin test was performed using an anti-human-β-globulin serum. When it was desired to make the test more sensitive, twenty volumes of serum were mixed with two volumes of complement and one volume of a 50 per cent suspension of red cells.

Owing to the limited amount of serum available, the samples obtained by heel prick from the eleven newborn infants were tested using the following dilutions: 1 in 2, 1 in 5, 1 in 10, 1 in 20 and 1 in 40. As controls, normal adult and cord sera were used at the same dilutions.

In this paper the amount of antibody is expressed as 'score' (Race and Sanger, 1958).

*Bacterial antibody test*

The indirect agglutination method was used (Neter, 1956). Suspensions of bacteria in buffered saline were heated for 1 hour at 100° and the supernatant was then recovered by centrifugation at 15,000 rev/min for 30 minutes. One volume of the supernatant was mixed with one volume of a 10 per cent suspension of group O red cells, previously washed with buffered saline, and incubated at 37° for 20 minutes. The 'modified' red cells were washed three times and resuspended in saline to make a final 5 per cent suspension.

The bacterial agglutinins were tested by adding one volume of the modified red cell suspension to each of a serial, doubling, dilution of the serum; after 1 hour at room temperature the deposited red cells were examined microscopically and the degree of agglutination scored.

*Treatment of sera with zymosan, other polysaccharides and simple sugars*

Aliquots of 1 ml of serum were added to 3 mg of zymosan; the mixtures were incubated for 1 hour at 0°, 17° and 37°; zymosan was maintained in suspension by frequent mixing. The supernatants were recovered by centrifugation at 15,000 rev/min, at the same temperature as used for the absorption stage. In the same way, fresh serum was treated with different amounts of potato starch.

In the experiments with dextran, inulin, D-glucose and L-fucose, two aliquots of 1 ml of serum (samples A and B) were used for each substance. After incubation at room temperature for 20 minutes the B series was dialysed at 0° for 2 hours against two changes of buffered saline; the A series was used undialysed. The n.i.c. activity in the treated sera was tested using human serum as a source of complement and an anti-β-globulin serum. Each sample was also titrated against sheep red cells at 37° for 1 hour and the degree of haemolysis scored.

*Inhibition* in vivo *of the n.i.c. antibody*

This experiment was performed by injecting a normal subject with 50 ml of a 6 per cent w/v solution of dextran in 0·9 per cent sodium chloride solution (Dextraven). Samples of blood were taken 3 days before the injection, 10 minutes before and 10 minutes after the injection; further samples were collected 1 hour, 3 hours, 1 day and 2, 5 and 8 days after the injection. The serum was separated as soon as possible and stored at −50°. The samples were all tested at the same time using the same source of complement and I-K serum.

The sera were also tested against sheep red cells at 37° and the degree of haemolysis scored.

*Absorption with bacteria*

Aliquots of 1 ml of serum were absorbed with different amounts of bacteria (wet weight) at 0° for 30 minutes. The serum was then centrifuged at 0° at 15,000 rev/min for 30 minutes and the precipitate discarded. In the same way aliquots of serum containing an anti-H agglutinin and a plant extract with anti-H specificity (*Ulex europaeus*) were absorbed with *E. coli* C3 and, as a control, *E. coli* A55.

*Heating at* 56° *and* 63°

One sample of serum from a group $A_1B$ donor, one serum containing an anti-$P_1$ agglutinin and one containing an incomplete anti-D antibody were used. Preliminary investigations using DEAE-cellulose chromatography and treatment of the serum with 2-mercaptoethanol (for methods see Adinolfi, Polley, Hunter and Mollison, 1962) had shown that the anti-$P_1$ agglutinin was a $\gamma$M-globulin and the anti-D antibody a $\gamma$G-globulin.

Aliquots of serum previously diluted with equal volumes of buffered saline were heated at 56° and 63° for 30 minutes, 1, 2 and 3 hours. The n.i.c. antibody was detected using horse serum as a source of complement and an I-K serum; the anti-$P_1$ by agglutination in saline at 12° and the anti-D antibody by the indirect antiglobulin test.

## RESULTS

### FREQUENCY OF THE N.I.C. ANTIBODY IN RANDOM ADULT DONORS; ITS RELATION TO THE ABO GROUPS

Using the test in which the ratio of serum to red cells was 20 to 1 the n.i.c. antibody was detected in forty-two out of the forty-five samples tested (93·3 per cent). The three sera apparently free of the antibody were retested using a ratio of serum to red cells of 40 to 1 and the antibody was then detected at a low titre in two of the samples. When the serum free of n.i.c. antibody was tested against sheep red cells at 37°, the degree of haemolysis was found similar to that of the serum used as controls. Thus failure to determine n.i.c. antibody was not due to any anticomplementary property of the serum.

The score of the antibody in the forty-five samples, in relation to the ABO blood groups is given in Fig. 1. The mean score of the antibody in the four ABO blood groups was calculated and the results are shown in Table 1; the titre of the n.i.c. antibody (expressed as the score) in the twenty-six AB, A and B subjects was compared with that in the nineteen group O donors and the data were analysed using the $t$ test (Snedecor, 1950). The value of $t$ obtained was 3·43 with 43 d.f.; $P<1$ per cent.

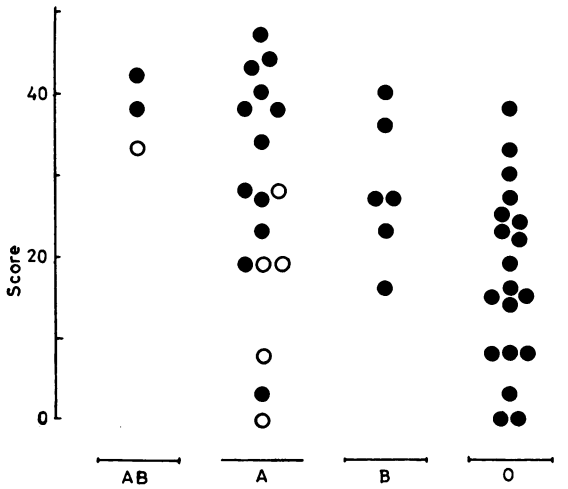


FIG. 1. Distribution of the n.i.c. antibody in forty-five sera from normal adult donors in relation to the ABO blood groups; O = $A_2B$ or $A_2$ subgroups.

TABLE 1

N.I.C. ANTIBODY IN FORTY-FIVE SERA FROM RANDOM ADULT DONORS*

| | Blood group | | | | |
|---|---|---|---|---|---|
| | $A_1B$ or $A_2B$ | $A_1$ | B | $A_2$ | O |
| Number tested | 3 | 12 | 6 | 5 | 19 |
| Antibody mean score | 37·6 | 31·6 | 28·5 | 14·6 | 15·5 |

* Mean score of n.i.c. in relation to the ABO blood groups.

The family data were analysed to see whether the amount of n.i.c. antibody in the serum was related to the ABO genotypes or to the ability to secrete A, B and H substances.

Sibs heterozygous for the ABO genes showed a considerable variation in the antibody titre; thus, the scores of the n.i.c. in five sibs of genotype $A_1O$ were 34, 5, 3, 39 and 36.

There was no difference between 'secretors' and 'non-secretors' with regard to the amount of antibody in the serum. This observation was confirmed testing six group O 'secretors' and six group O 'non-secretors'; thus the mean scores of the antibody were respectively 45·5 and 47·8.

## MATERNAL AND CORD SERA

The n.i.c. antibody was present in nineteen out of twenty-three maternal sera (82·6 per cent) and in twenty out of twenty-three cord sera (86·9 per cent). The mean score of the antibody, calculated only for those samples in which antibody was present, was 31·7 for the nineteen maternal samples and 26·5 for the twenty cord sera. Fig. 2 shows the score of the antibody in each maternal sample compared with that in the relevant cord serum. In twelve out of the seventeen pairs of maternal and cord sera in which the antibody was present in both samples, the antibody had the same score or the difference did not exceed

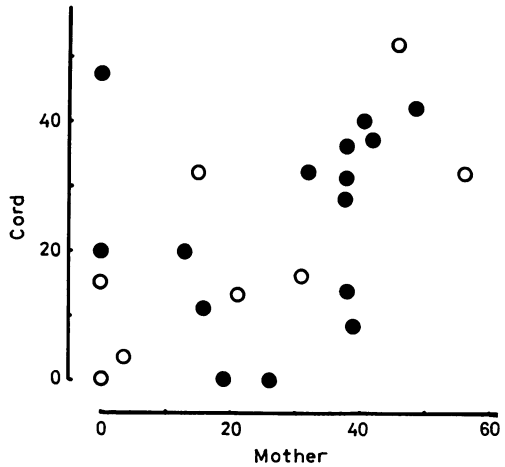


FIG. 2. N.i.c. antibody in twenty-three maternal sera and corresponding cord sera; results are expressed as scores. ● = Mother and newborn infant of same ABO blood group; ○ = mother and newborn infant of different ABO blood group.

10 units; in one case the antibody had a definitely higher score in the maternal sample; in four cases the antibody had a higher score in the cord serum.

TABLE 2

N.I.C. ANTIBODY IN TWENTY-THREE PAIRS OF MATERNAL AND CORD SERA

| | Mother | Cord | Observed | Expected* |
|---|---|---|---|---|
| (i) | Present | Present | 17 | 16·6 |
| (ii) | Present | Absent | 2 | 2·5 |
| (iii) | Absent | Present | 3 | 3·4 |
| (iv) | Absent | Absent | 1 | 0·5 |

* The expected frequency was calculated assuming that the presence of the antibody in cord serum was independent of its presence in the corresponding maternal sample.

Table 2 gives the distribution of the n.i.c. antibody in twenty-three pairs of sera in relation to the four possible combinations in which it may occur. The observed frequency for each of the four possible combinations was compared with the expected frequency calculated on the assumption that the presence of the antibody in the mother's serum did not influence its presence in the infant's serum. In three cases, as expected on statistical grounds, the antibody was present in the cord serum and absent from the corresponding maternal serum.

## THE N.I.C. ANTIBODY IN NORMAL INFANTS

The antibody was present in the serum of all the eleven newborn infants whose blood was collected by heel prick (Fig. 3). The score of the antibody ranged from 3 to 45 score units and was similar to that of the adult and cord sera used as controls. The score of the antibody in one infant bled three times within 6 weeks showed no significant change.

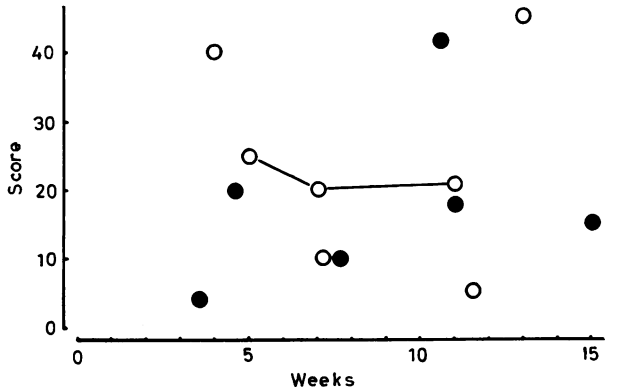


FIG. 3. N.i.c. antibody in eleven newborn infants bled by heel prick. ● = Premature infants. The three observations joined by a line were made on the same infant.

The antibody was present in the serum of six of the seven infants bled three times with intervals of about 5 weeks between bleedings (Fig. 4). The antibody had a constant score in two infants; in another three infants the score of the second and third samples was a little higher than that in the first sample; in the remaining case the antibody rose progressively from 22 to 43 'score' units between the first and the third samples.

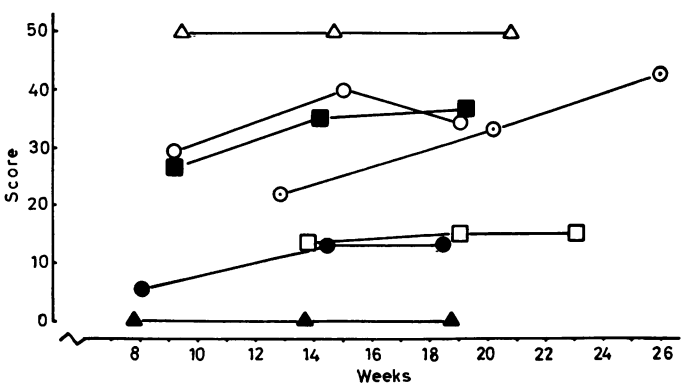


FIG. 4. N.i.c. antibody in seven newborn infants bled by venepuncture at intervals of about 5 weeks.

### N.I.C. ANTIBODY IN PATIENTS WITH HYPOGAMMAGLOBULINAEMIA

Table 3 shows the results of testing fourteen sera from patients with hypogammaglobulinaemia. The antibody was found in all but one serum (No. 13). Owing to the limited amount of serum available the antibody could be titrated only in one serum stored at −20° for 2 years and in six sera stored at −50° for a few weeks after being collected. The titre of the n.i.c. in these sera was similar to that of the normal sera used as controls. Anti-A, anti-B and anti-I antibodies were absent from all sera; sera No. 3 and No. 4 were proved to be free of bacterial agglutinins against *E. coli* C3, *E. coli* A55, *Salmonella enteritidis* variety Danysz, *Staphilococcus aureus* 80, and *Pneumococcus* type XIV.

TABLE 3

N.I.C. IN SERUM OF PATIENTS WITH HYPOGAMMAGLOBULINAEMIA SYNDROME*

| No. | Initials | γ-Globulins | | | Blood group | Anti-A | Anti-B | Anti-I | n.i.c.‡ |
|---|---|---|---|---|---|---|---|---|---|
| | | γG (mg/100 ml) | γA† (% of normal serum conc.) | γM† | | | | | |
| 1 | H.H. | 6 | 2 | 62 | A | ... | nil | nil | 32 |
| 2 | D.H. | 17·5 | 3 | 100 | A | ... | nil | nil | 16 |
| 3 | S.S. | 12·8 | 0·5 | 17 | O | nil | nil | nil | 64 |
| 4 | T.W. | 25 | 0·2 | 0 | B | nil | ... | nil | 64 |
| 5 | A.A. | 300 | ... | ... | O | nil | nil | nil | 32 |
| 6 | A.W. | 20 | 8 | 6 | B | nil | ... | nil | 16 |
| 7 | C.W. | 140 | 3 | 6 | A | ... | nil | nil | 16 |
| 8 | J.A. | 40 | 2 | 6 | O | nil | nil | nil | ++ |
| 9 | J.A. | 2·5 | 0 | 25 | O | nil | nil | nil | + |
| 10 | A.G. | 25 | 0 | 300 | AB | ... | ... | nil | ++ |
| 11 | M.B. | 80 | ... | ... | A | ... | nil | nil | ++ |
| 12 | J.C. | 30 | 2 | 200 | A | ... | nil | nil | + |
| 13 | B.W. | 40 | 4 | 18 | O | nil | nil | nil | − |
| 14 | E.H. | 200 | 0 | 0 | A | ... | nil | nil | +++ |

* The first six sera were stored at −50° for only a few weeks; the others were stored at −20° for periods between 2 and 6 years.

† The concentrations of the γA and γM globulins are expressed as percentages of the amounts in 'standard' normal sera.

‡ The n.i.c. antibody was titrated in seven samples (titre expressed as reciprocals). The degree of agglutination of the other seven sera was compared with that of a normal serum which gave a +++ agglutination.

## REACTION OF N.I.C. WITH SOME POLYSACCHARIDES AND SIMPLE SUGARS

Samples of serum absorbed at 0°, 17° and 37° with different batches of zymosan type A (1 ml serum per 3 mg zymosan) were proved to be free of n.i.c. antibody. The antibody was also absorbed with two batches of potato starch; thus 0·3 g (wet weight) of potato starch incubated at 37° for 1 hour with 1 ml of serum completely removed the n.i.c. activity present in a serum containing the antibody at a titre of 1 in 64. The antibody could also be inhibited by mixing 1 ml of serum with the following amounts of polysaccharides and simple sugars: 60 mg dextran (Dextraven, Bengers Lab. Ltd), 100 mg inulin, 100 mg D-glucose and 100 mg L-fucose.

When 50 ml of a 6 per cent solution of dextran (Dextraven) was injected intravenously into a normal subject the score of the n.i.c. antibody fell from 30 to 3 units within 10 minutes and the pre-injection score was not regained for more than 2 days (see Fig. 5).

In all the experiments in which polysaccharides and simple sugars were used, controls were included to provide evidence that the negative results obtained in testing the n.i.c. antibody in the treated sera were not due to an interference between the substances used and complement. Thus, for example, the n.i.c. activity in the samples collected after the injection of dextran, was tested before and after adding fresh complement to the sera (see Methods); the same sera, and one sample of serum taken before the injection, were also tested against sheep red cells at 37° and the degree of haemolysis compared; thus it was found that whereas the dextran solution reduced the titre of the n.i.c. antibody, it did not alter appreciably the titre of complement.

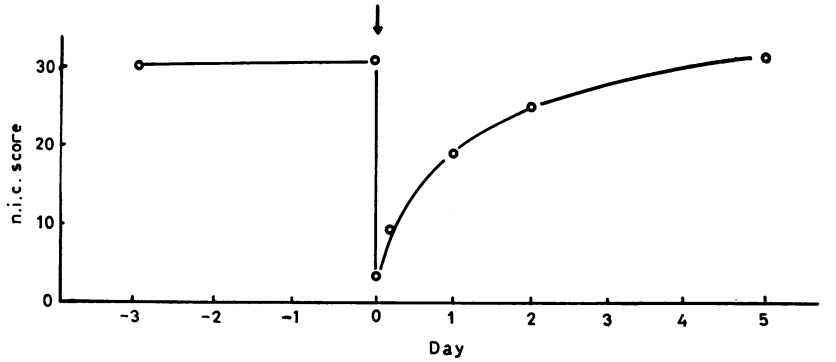


FIG. 5. Titre of n.i.c. antibody before and after an intravenous injection of 50 ml of a 6 per cent solution of dextran (Dextraven).

*Absorption with bacteria*

The n.i.c. antibody of three normal adult sera and two sera of patients with hypogammaglobulinaemia was completely removed after one absorption with *E. coli* C3 whereas absorption with the other bacteria was ineffective. Preliminary experiments showed that the nutrient agar used in growing *E. coli* C3 did not inhibit the antibody.

TABLE 4

EFFECTS OF ABSORPTION OF SERA UPON N.I.C. ANTIBODY

| | | Normal serum | | Serum of a patient with hypogammaglobulinaemia | |
|---|---|---|---|---|---|
| | | n.i.c. | Anti-*E. coli* C3 | n.i.c. | Anti-*E. coli* C3 |
| (i) | Untreated serum | +++ | +++ | +++ | − |
| (ii) | Heated at 56° for 30 minutes | + | +++ | ... | ... |
| (iii) | Absorbed at 0° with human red cells | − | +++ | − | − |
| (iv) | Treated with H substance | − | +++ | − | − |
| (v) | Absorbed with *E. coli* C3 | − | − | − | − |
| (vi) | Absorbed with *E. coli* A55 | +++ | +++ | +++ | − |
| (vii) | Absorbed with *Staphilococcus aureus* 80 | +++ | +++ | +++ | − |
| (viii) | Absorbed with *Salmonella enteritis* | +++ | +++ | +++ | − |
| (ix) | Absorbed with *Pneumococcus* type XIV | +++ | +++ | +++ | − |

Table 4 gives the results of testing the n.i.c. antibody and *E. coli* C3 agglutinin in a normal serum and a serum of a patient with hypogammaglobulinaemia before and after absorption with bacteria. The two sera were also tested after heating at 56° for 30 minutes, after absorption with group O red cells at 0° for 2 hours and after adding H substance present in the saliva of a 'secretor'. From the above results it appears that *E. coli* agglutinin is not inhibited by H substance. On the other hand it was observed that these bacteria did not absorb the anti-H agglutinin present in a human serum or a plant agglutinin with anti-H specificity extracted from the *Ulex europaeuo.*

*Effect of heating at* 56° *and* 63°

Fig. 6 shows the activity of the n.i.c. antibody and of the anti-D and anti-$P_1$ antibodies after heating at 56° and 63° for different periods of time. Heating at 56° for 30 minutes

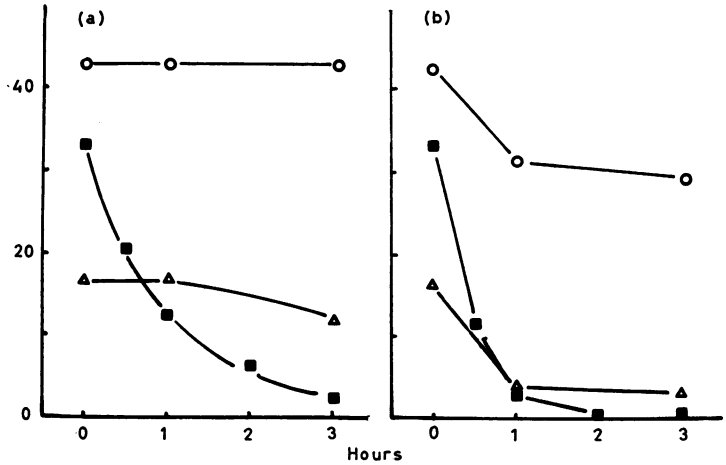


FIG. 6. Effect of heating anti-D (○), anti-$P_1$ (△) and n.i.c. (■) antibodies at (a) 56° and (b) 63°; titre are expressed as scores.

produced a marked fall in the titre of the n.i.c. antibody; this was completely destroyed after 3hours; by contrast heating at 56° for 3 hours did not alter the titre of the incomplete anti-D, whereas the titre of the anti-$P_1$ agglutinin was only slightly reduced. When the experiment was repeated at 63°, both n.i.c. and anti-$P_1$ antibodies were destroyed after 1 hour; after 3 hours at 63° the titre score of the anti-D antibody fell from 43 to 31 units.

## DISCUSSION

The n.i.c. antibody has a specificity closely related to anti-H; thus it reacts more strongly with group O red cells than with $A_1B$ cells; the serum of group $A_1B$ donors usually contains more antibody than the serum of group O donors and the reactions are inhibited by the saliva of 'secretors' but not by the saliva of 'non-secretors' (Crawford, Cutbush and Mollison, 1953). Furthermore it has been shown that the antibody does not react with Bombay red cells (Mollison, 1961).

The present results confirm that the titre of the antibody is higher in $A_1B$ donors than in group O donors (Fig. 1 and Table 1) and show that the difference between group O donors and those of other groups is highly significant ($t = 3{\cdot}43$; d.f. $= 43$; $P<1$ per cent). It thus appears that the amount of the antibody in normal sera is inversely related to the amount of H substance on the red cells, as follows: $A_1B > A_1 > B > A_2B > A_2 > O$.

In view of this relation between the titre of the n.i.c. antibody and the ABO phenotypes, preliminary investigations were carried out on a limited number of families to see whether any difference in the amount of the antibody in the serum could be detected in subjects homozygous or heterozygous for ABO genes. The family data were also analysed to see whether the ability to secrete A, B and H substances had any influence on the amount of antibody. Clarke, McConnell and Sheppard (1960) have demonstrated that group O subjects secrete more H substances than group A donors, who, in turn, secrete more than groups B and AB. They have shown also that the amount of A, B and H substances secreted by an individual is in part inherited and that probably there is a difference between homozygous and heterozygous group A and B donors with regard to the amount

of H substance secreted in their saliva. In view of this evidence and the fact that the n.i.c. antibody has anti-H specificity, it seemed possible that the titre of the antibody in individuals might depend on an interaction between the ABO genotype and the 'secretor' status.

The present data showed that the titre of the antibody was variable in sibs of the same ABO genotype and that there was no relation between the amount of the antibody in serum and the ability to secrete A, B and H substances.

It appears that the n.i.c. antibody is produced by infants before birth; thus in three cases the antibody was detected in the serum of infants whose mothers lacked the antibody. Furthermore, n.i.c. antibody was found in almost all infants between 4 and 26 weeks of age and in many cases its titre remained approximately constant during this period. If the antibody had been of maternal origin ($\gamma$G-globulin) a temporary disappearance of the antibody or fall in titre after birth would have been expected. The antibody is apparently not a $\gamma$G-globulin (see also Adinolfi *et al.*, 1963) and the observation that it is present in the serum of thirteen out of fourteen patients with hypogammaglobulinaemia (Table 3) gives further support to this view.

The n.i.c. antibody differs from blood group antibodies also in its thermolability; thus it is relatively heat-labile at 56° and is rapidly destroyed at 63°; in this respect the n.i.c. antibody behaves differently from incomplete anti-D antibody ($\gamma$G-globulin) and anti-$P_1$ agglutinin ($\gamma$M-globulin). On the basis of the above results striking similarities may be drawn between n.i.c. and properdin; properdin, as well as n.i.c. is present in cord serum (Koch, Schultze and Schwick, 1958) in the serum of patients with hypogammaglobulinaemia (Barandum and Isliker, 1956) and it is thermolabile (Pillemer, Blum, Lepow, Ross, Todd and Wardlaw, 1954). Furthermore, properdin does not react with anti-$\gamma$G globulin serum (Hinz, Wedgewood, Todd and Pillemer, 1960).

In view of these similarities further investigations were carried out to see whether n.i.c. antibody and properdin had other serological characteristics in common. It has been shown that zymosan, potato starch, dextran and inulin react with properdin (Pillemer, Schoemberg, Blum and Wurz, 1955; Lepow, Pillemer, Schoemberg, Todd and Wedgewood, 1959; Turk, 1959) and in the present work these polysaccharides were found to remove n.i.c. antibody from human serum. With regard to the absorption with polysaccharides and simple sugars the antibody resembles the properdin-like factor described by Turk (1959) in rabbits. Nevertheless n.i.c. antibody and properdin differ in many respects; for example whereas the n.i.c. antibody is absorbed by zymosan at 0° as well as 37°, properdin is absorbed only at 17° or higher temperature. Very recently a properdin-like factor which is absorbed by zymosan at 0° has been described by Blum (1964) but it is not yet known whether this factor is related to n.i.c. antibody.

N.i.c. antibody also differs from properdin in its reaction with bacteria; thus whereas a wide range of bacteria absorbs properdin (Pillemer *et al.*, 1955) only *E. coli* C3 was found to absorb n.i.c. antibody. Incidentally, although *E. coli* C3 bacteria inhibited the antibody, these bacteria did not react with a human anti-H antibody or with a plant extract with anti-H specificity (*Ulex europaeus*) and H substance present in the saliva of a secretor did not inhibit anti-*E. coli* C3 agglutinins (Table 4).

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