J. Hyg., Camb. (1982), **89**, 329–334
Printed in Great Britain

The postnatal acquisition of factors which affect the influenza haemagglutination-inhibition test

By P. C. A. GRINT, S. ARGENT and R. B. HEATH

Virology Department, St Bartholomew's Hospital, West Smithfield, London, EC1A 7BE

(*Received* 26 *March* 1982; *accepted* 2 *May* 1982)

SUMMARY

Levels of maternally transferred antibodies against the surface antigen of the A/Texas/1/77 strain of influenza virus showed the expected decline during infancy when measured by complement fixation (CF). However, this decline was not observed when these antibodies were measured by haemagglutination-inhibition (HI). It has been postulated that this discrepancy is due to the acquisition, in the early days of life, of non-specific serum factors which increase the HI activity of sera. The levels of these factors were determined indirectly by calculating HI:CF ratios and it was shown that the factors are rapidly acquired by children between the fifth and twentieth week of life.

INTRODUCTION

Antibodies of the IgG class that are transferred *in utero* to the fetus are known to be selectively concentrated on the fetal side of the circulation (Kohler & Farr, 1966). Antibodies against influenza viruses, however, appear to be anomalous in that they are either not concentrated in this way or appear in higher concentrations in the maternal rather than the fetal circulation (Masurel *et al.* 1978; Sarateanu, Ehrengut & Fofana, 1980). We have recently confirmed these findings by showing that antibodies against a standard strain of influenza A/Texas/1/77 behave in this same unusual way when assayed by haemagglutination–inhibition (HI) and we have suggested that this anomaly results from the activity of non-specific inhibitors or similar factors that are normally present to higher titre in adults' sera than in cord sera (Griffiths *et al.* 1982).

In this study we have attempted to find out at what time after birth these non-specific factors appear in infants' sera.

MATERIALS AND METHODS

Serological tests

The influenza virus used in this study was the A/Texas/1/77 (H3N2) strain. Details of the preparation of this virus for use as antigen in both the HI test and a complement fixation (CF) test using viral antigen, as well as details of the procedures used, have been described in a previous paper (Griffiths *et al.* 1982).

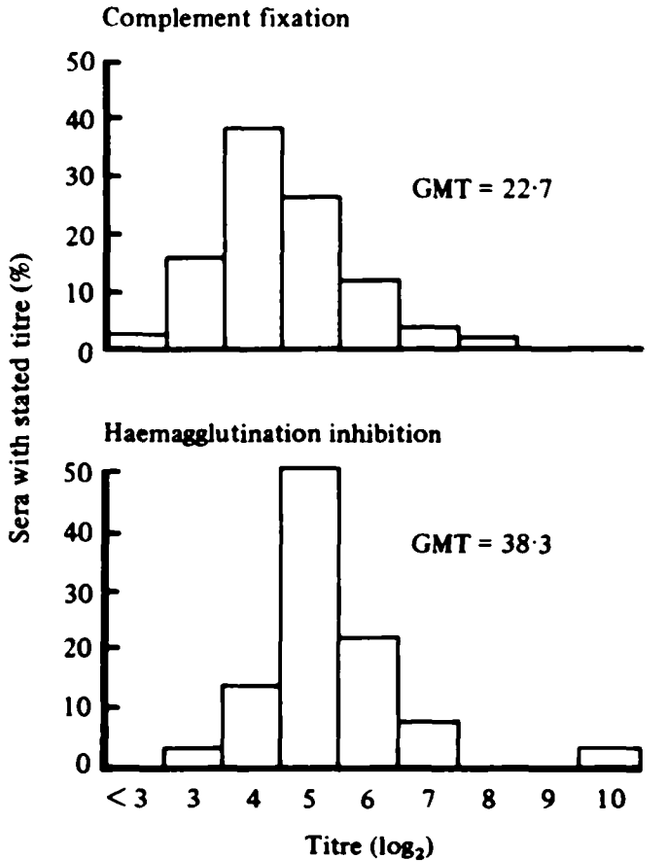

Fig. 1. The distribution of levels of antibody against the A/Texas/1/77 strain of influenza virus in 50 adult sera as determined by CF and HI.

Selection of sera

The following sera were tested by both HI and CF: (*a*) 25 cord sera obtained from unselected deliveries at this hospital during early 1981. (*b*) 75 sera obtained from babies in the first year of life that had been submitted to the laboratory for a variety of diagnostic purposes during the years 1980 and 1981 and (*c*) 50 sera obtained from patients booking at our antenatal clinic during 1981. These antenatal patients were of approximately the same age, and therefore likely to have had the same exposure to influenza, as the mothers of the children who had donated the sera referred to in (*a*) and (*b*) above.

RESULTS

Antibody titres of adult sera

The 50 sera obtained from antenatal patients were titrated against the influenza A/Texas strain by both HI and CF and the results are shown in Fig. 1. All the sera were shown to contain antibody by HI and all but one by CF. Haemagglutination-inhibition proved to be the more sensitive test with a modal titre of 32 (5 $\log_2$ units) and a geometric mean titre (GMT) of 38·3 whereas the modal CF titre was 16 (4 $\log_2$ units) and the GMT of these sera by CF was 22·7.

The ratio of the HI titre to the CF titre for each individual serum was calculated and the distribution of these HI : CF ratios, calculated as $\log_2$ HI titre $-$ $\log_2$ CF titre,

Table 1. *Ratio of HI:CF titres of antibody against the A/Texas/1/77 virus in the sera of 50 adult patients*

HI:CF ratios	No. of sera
−2	3
−1	4
0	11
+1	18
+2	12
+3	2

Mean HI:CF ratio ($\log_2$ HI titre − $\log_2$ CF titre) = 0·76.

is shown in Table 1. It can be seen that the values ranged from −2 to 3 with a mean of 0·76.

Antibody titres of cord and infants' sera

The 25 cord sera and 75 infants' sera were next tested by HI and CF and the distribution of the titres of these sera with age is shown in Fig. 2. The sequential levels of CF activity followed the pattern to be expected for maternally transferred antibodies and fell gradually but consistently during infancy. There were, however, sera from two children aged 10 months and 1 year who had relatively high CF titres and it is possible that these two children had recently been infected with an influenza A virus. It can, however, be seen from Fig. 2. that the distribution of the HI titres of these sera contrasted markedly with the distribution of titres obtained by CF. There was no obvious decline in the HI antibody titres with age.

These results suggest that the maternally transferred antibodies against this influenza virus, although declining in infancy, have their activity rapidly either enhanced or added to in the early months of life by the acquisition or development of non-specific serum factors. Since it is probable that CF gives a true measure of antibody levels and that the HI activity of these antibodies is increased to varying degrees by the serum factors, then an indirect measure of these factors present in an individual serum will be given by the ratio of HI titre to CF titre. The ratios for the 25 cord and 75 infants' sera were calculated and their distribution with age is shown in Fig. 3. The HI:CF ratios of the 25 cord sera were all low, having values $\leqslant 1{\cdot}0$. Thereafter, although the ratios varied considerably there was an obvious increase in their value up to the age of about 20 weeks after which they tended to plateau.

A more detailed analysis of these results is shown in Table 2. The mean of the HI:CF ratios of the sera obtained from neonates aged less than 5 weeks was higher than the corresponding ratio of the cord sera but the difference was not statistically significant. However, the means of the HI:CF ratios of the sera obtained from each of the groups of children aged 5–19 and $\geqslant 20$ weeks were both significantly higher than the mean ratio of the cord sera. It was of interest to note that the mean HI:CF ratio of 2·55 obtained with the sera of children older than 20 weeks were significantly greater ($t = 7{\cdot}7$, $P < 0{\cdot}001$) than the corresponding ratio of 0·76 obtained with the sera from the 50 adult women (see Table 1).

https://doi.org/10.1017/S0022172400070868 Published online by Cambridge University Press

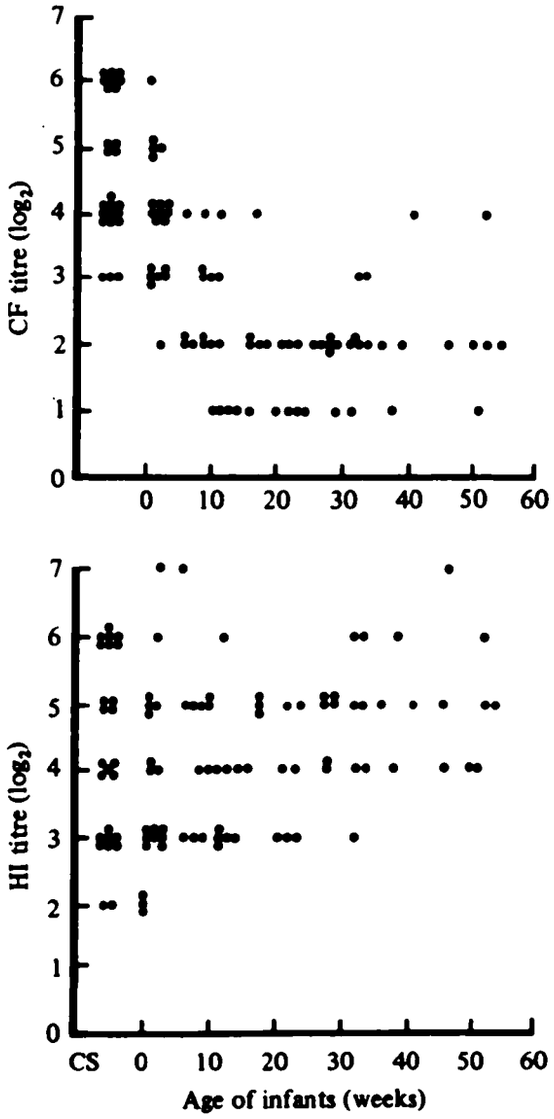

Fig. 2. Levels of antibody against the A/Texas/1/77 strain of influenza virus in 25 cord and 75 infants' sera as determined by CF and HI. (CS = cord sera.)

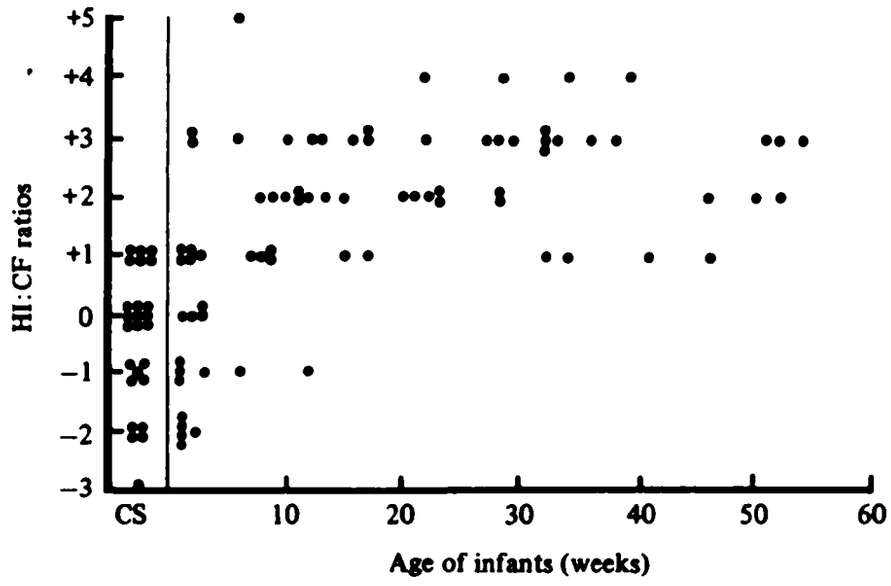

Fig. 3. Ratios of the HI to CF titres of antibodies against the A/Texas/1/77 strain of influenza virus present in 25 cord and 75 infants' sera. (CS = cord sera. HI:CF ratios calculated as $\log_2$ HI titre − $\log_2$ CF titre.)

https://doi.org/10.1017/S0022172400070868 Published online by Cambridge University Press

Table 2. *Analysis of the ratios of the HI to CF titres of antibodies present in cord and infants' sera against the influenza A/Texas/1/77 virus*

Age group (Weeks)	No. of sera	Mean HI:CF ratio	Difference relative to cord sera	Mann–Whitney U value
Cord sera	25	−0·4	—	—
0–4	20	−0·15	0·25	237 ($P>0·5$)
5–19	24	1·92	2·32	53 ($P<0·001$)
⩾20	31	2·55	2·95	12 ($P<0·001$)

It is clear from these results that the serum factors which affect the influenza HI test are rapidly acquired after birth and that with most children the levels of these factors appear to be equal or greater than the levels in sera from unrelated adults.

DISCUSSION

Although it is well established that maternal antibodies of the IgG class are preferentially concentrated by the fetus, it has frequently been observed that HI antibodies against influenza viruses are either present in the same concentration in cord and maternal sera or are present in higher concentration in the latter (Masurel *et al.* 1978 and Sarateanu, Ehrengut & Fofana, 1980). We have recently confirmed this anomalous distribution of HI antibodies against influenza viruses in cord and maternal sera (Griffiths *et al.* 1982). However, the expected higher concentration of these antibodies was found in the cord sera when these were assayed by CF and single radial haemolysis tests. We therefore postulated that the apparent high concentration of HI antibody present in maternal sera was due to the presence of non-specific factors that were absent or of lower titre in the cord sera.

This hypothesis was supported by the results described here. The decline in the levels of maternally transferred antibody in infants' sera as demonstrated by CF followed the expected pattern (Sato *et al.* 1979) but no obvious decline was apparent when the same sera were tested by HI. This is probably due to the fact that the activity of the falling levels of antibody was being increasingly enhanced or added to by the acquisition of non specific factors in the serum in the early months of life. It has further been shown that these factors, as measured by HI:CF ratios, appear in the serum of young children from approximately the 5th to the 20th week of life.

The nature of these rapidly acquired serum factors, that are not completely removed by pre-treatment with receptor-destroying enzyme, is not known. Whether they are identical or similar to one or more of the previously described α, β or γ non-specific inhibitors of influenza (Francis, 1947; Hirst, 1942; Chu, 1951; Shimojo *et al.* 1958) or even cofactors necessary for the reaction of specific antibodies with influenza described by Styk (1961), remains to be determined. It will be of further interest to find out if these factors have neutralizing properties or are able to enhance the neutralizing activity of antibodies against influenza viruses.

The presence of these non-specific factors in serum is partly responsible for the well-known unreliability of the HI test for determining susceptibility to influenza, and why tests such as single radial haemolysis are preferred for this purpose.

REFERENCES

CHU, C. M. (1951). The action of normal mouse serum on influenza virus. *Journal of General Microbiology* **5**, 739.

FRANCIS, T. (1947). Dissociation of haemagglutinating and antibody measuring capacities of influenza virus. *Journal of Experimental Medicine* **85**, 1.

GRIFFITHS, P. D., BERNEY, S. I., ARGENT, S. & HEATH, R. B. (1982). Antibody against viruses in maternal and cord sera: specific antibody is concentrated by the fetus. *Journal of Hygiene* **89**, 303.

HIRST, G. K. (1942). The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *Journal of Experimental Medicine* **75**, 49.

KOHLER, P. F. & FARR, R. S. (1966). Elevation of cord over maternal IgG immunoglobulin: evidence for an active placental IgG transport. *Nature* **210**, 1070.

MASUREL, N., DEBRUIJNE, J. I., BEUNINGH, H. A. & SCHOUTEN, H. J. A. (1978). Haemagglutination-inhibition antibodies against influenza A and influenza B in maternal and neonatal sera. *Journal of Hygiene* **80**, 13.

SARATEANU, D. E., EHRENGUT, W. & FOFANA, Y. (1980). Influenza and corona-virus antibodies in the newborns and their mothers in Mali. *Tropenmedizin and Parasitologie* **31**, 399.

SATO, H., ALBRECHT, P., REYNOLDS, D. W., STAGNO, S. & ENNIS, F. A. (1979). Transfer of measles, mumps and rubella antibodies from mothers to infant. *American Journal of Diseases of Children* **133**, 1240.

SHIMOJO, H., SIGIURA, A., AKAO, T. & ENOMOTO, C. (1958). Studies of a non-specific inhibitor of influenza A/Asian/57/Virus. *Bulletin of the Institute of Public Health, Tokyo* **7**, 219.

STYK, B. (1961). Cofactor and specific antibodies against influenza viruses. I. Method of cofactor titration. Cofactor content of various animal sera. *Acta Virologica* **5**, 334.