CIRCULATING ANTIBODIES TO COW'S MILK PROTEINS AND GLUTEN IN THE NEWBORN

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Recent studies have shown that in certain diseases of the alimentary tract abnormally high concentrations of circulating antibodies to cow's milk proteins may occur. Thus Taylor and Truelove (1961) found that such antibodies were more likely to be present in high titres in patients with ulcerative colitis than in healthy subjects, and this has been confirmed by other workers (Gray, 1961; Davidson, 1961). Similarly, patients with coeliac disease and idiopathic steatorrhoea are much more likely to have circulating antibodies in high titres to cow's milk proteins and to partly proteolysed wheat gluten (Taylor *et al.*, 1961).

These high antibody titres might be an expression of pre-existing states of hypersensitivity to common dietary proteins, which may serve as aetiological factors in these diseases, or they might indicate that in certain alimentary diseases abnormal absorption of such proteins occurs, with enhanced antibody formation.

Acheson and Truelove (1961) have found that patients with ulcerative colitis are more likely to have been bottle-fed during the first few weeks of life than healthy matched controls. This suggests that there may be a relationship between early exposure to potentially antigenic material and later development of a disease which could possibly be caused wholly or in part by a disturbance of normal immunological response. That milk proteins may be involved in such a way is strongly supported by clinical observations which have shown that some patients with ulcerative colitis become symptom-free when put on a milk-free diet and relapse in a few days or weeks after reintroduction of cow's milk into the diet (Andresen, 1942; Rowe, 1942; Rider *et al.*, 1960; Truelove, 1961).

These considerations led us to study the levels of circulating antibodies to cow's milk proteins and wheat gluten in various age-groups, and we report here our findings in the cord blood of newly delivered infants and in the blood of their mothers.

Materials and Methods

Specimens of cord blood from 100 babies were collected from the labour wards of the Radcliffe Infirmary, and specimens of blood from the 100 corresponding mothers were obtained by venepuncture within 24 hours of the time of delivery. The sera were stored at -20°C .

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The test employed was the coated tanned red-cell test of Boyden (1951) as modified by Witebsky and Rose (1956) and Gunther *et al.* (1960). The test was read and scored as described previously (Taylor and Truelove, 1961). With each individual antigen, all the sera of mothers and babies were tested as a single batch on the same day to minimize technical errors.

As in our previous studies, purified cow's milk proteins (casein, α -lactalbumin, and β -lactoglobulin) were used as the milk antigens. A soluble product of proteolysed gluten (fraction III of Frazer *et al.*, 1959) was used as the gluten antigen.

Results

Table I and Fig. 1 show the overall results for the 100 pairs of sera. It is easily seen from the Chart that the comparative distributions in mothers and babies of titres of circulating antibodies vary according to the particular antigen. With casein and α -lactalbumin the frequency distributions of levels of circulating antibody are approximately the same in mothers and babies. With β -lactoglobulin and especially with gluten fraction III the distributions are different. Moreover, the differences are in a direction which at first sight appears surprising—namely, that the infants on average possess higher levels of circulating antibodies than the mothers.

TABLE I.—Titres of Serological Reactions in 100 Specimens of Maternal and 100 Corresponding Specimens of Cord Blood

Antigen	Specimen	Serological Reaction					Total	Statistical Significance
		Neg.	1/20	1/200	1/2,000	1/20,000		
Casein	Maternal	12	44	29	9	6	100	Combining the values for titres of 1/2,000 and 1/20,000, $\chi^2 = 3.9$, $n=3$, $P > 0.05$. Not significant
	Cord	9	58	22	9	2	100	
α -Lactalbumin	Maternal	14	47	31	6	2	100	Combining the values for titres of 1/2,000 and 1/20,000, $\chi^2 = 7.0$, $n=3$, $P > 0.05$. Not significant
	Cord	24	39	22	7	8	100	
β -Lactoglobulin	Maternal	60	25	10	3	2	100	Combining the values for titres of 1/2,000 and 1/20,000, $\chi^2 = 15.3$, $n=3$, $P < 0.01$. Significant
	Cord	34	37	24	5	—	100	
Gluten fraction III	Maternal	68	30	2	—	—	100	Combining all values for titres of 1/200 and above, $\chi^2 = 60.7$, $n=2$, $P < 0.001$. Highly significant
	Cord	15	67	17	1	—	100	

There is no close correspondence between the titres of these antibodies in the sera of mother and child. This can be seen when the data are set out in contingency tables as in Table II. The considerable measure of difference which often exists between mother and child is obvious. Statistically, there are feebly positive coefficients of correlation with three of the antibodies. A significantly positive coefficient of correlation is present only in the case of α -lactalbumin, but even with this there are many examples of poor correspondence between mother and child.

Further Examination of Data.—We have further examined the data and have made additional tests to exclude the possibility of extraneous factors being responsible for the differences between mothers and babies. Some of the sera obtained from cord blood showed evidence of haemolysis. Exclusion of the results

obtained with these sera and with the sera of the corresponding mothers left the results for the remaining 76 pairs of sera essentially similar to those of the full 100 pairs.

Frequency distribution of serological reactions at various titres in mothers and their babies in relation to four dietary antigens. (Coated tanned red-cell test.)

TABLE II.—Correlation Between the Serological Reactions in Maternal and Cord Blood

Maternal Blood	Cord Blood						Statistical Significance
	Neg.	1/20	1/100	1/2,000	1/20,000	Total	
Casein							
Neg. ..	2	6	4	—	—	12	$r = +0.075$ $P > 0.05$ Not significant
1/20 ..	4	28	6	4	2	44	
1/100 ..	2	15	9	3	—	29	
1/2,000 ..	1	5	2	1	—	9	
1/20,000 ..	—	4	1	1	—	6	
Total	9	58	22	9	2	100	
α -Lactalbumin							
Neg. ..	7	5	—	1	—	14	$r = +0.305$ $P < 0.01$ Significant
1/20 ..	13	20	8	5	1	47	
1/100 ..	4	11	10	2	4	31	
1/2,000 ..	—	1	3	—	2	6	
1/20,000 ..	—	2	—	—	—	2	
Total	24	39	22	7	8	100	
β -Lactoglobulin							
Neg. ..	22	24	12	2	—	60	$r = +0.110$ $P > 0.05$ Not significant
1/20 ..	9	7	6	3	—	25	
1/100 ..	2	3	5	—	—	10	
1/2,000 ..	—	3	—	—	—	3	
1/20,000 ..	1	—	1	—	—	2	
Total	34	37	24	5	—	100	
Gluten Fraction III							
Neg. ..	9	48	10	1	—	68	$r = +0.021$ $P > 0.05$ Not significant
1/20 ..	6	18	6	—	—	30	
1/100 ..	—	1	1	—	—	2	
1/2,000 ..	—	—	—	—	—	—	
1/20,000 ..	—	—	—	—	—	—	
Total	15	67	17	1	—	100	

The sex of the baby had no influence on the results. Likewise, the parity of the mother appeared to be of no consequence.

Some of the pregnancies were abnormal, being premature, post-mature, or associated with pre-eclamptic toxæmia. When the results for these are considered it appears that this abnormal group was more prone to show high titres of circulating antibodies than the normal group, although the relationship between mother's blood and cord blood was similar to that found in normal pregnancies. The whole question of the levels of circulating antibodies in abnormal pregnancies is an issue which deserves further study.

Additional Tests.—Repeat tests with these antigens have shown close reproducibility of results. In order to test the specificity of the reactions, inhibition tests were performed with casein, β -lactoglobulin, and gluten fraction III. Specific inhibition was demonstrated. In the case of α -lactalbumin, inhibition tests were not performed because the antigen was scarce. Maternal sera which showed no reactions to casein, to β -lactoglobulin, and to gluten fraction III at a titre of 1/20 were tested at titres of 1/4 and 1/10 against the appropriate antigen (Table III). The majority of the sera showed reactions at a titre of 1/10, and all showed reaction at a titre of 1/4 apart from six sera which showed no reaction to lactoglobulin. Inhibition tests showed that these reactions at low titre were specific.

TABLE III.—Results of Testing Maternal Sera at Lower Titres than were Employed in the Original Test

Antigen	No. of Maternal Sera Negative at 1/20	No. Negative at 1/10	No. Negative at 1/4
Casein ..	12	2	0
β -Lactoglobulin ..	60	10	6
Gluten fraction III ..	30*	3*	0*

* Only 30 of the 68 maternal sera negative at 1/20 were tested.

Discussion

The present study appears to be the first in which titres of circulating antibodies to purified cow's milk proteins and to gluten fraction III have been studied in maternal and cord blood, although the presence in cord blood of circulating antibodies to casein or to whole milk proteins has already been demonstrated (Berger and Bauer, 1959; Gunther *et al.*, 1960). With the present data on 100 mothers and their infants, the relationship between the findings in maternal and cord blood can be examined statistically for each of the antigens studied.

A conspicuous feature is the lack of close correlation between the titres of maternal and cord blood, although this varies from antigen to antigen, there being a significant positive correlation with α -lactalbumin but not with the other antigens. Another conspicuous feature is that higher titres of circulating antibody are more likely to be present in cord blood than in maternal blood in the case of β -lactoglobulin and gluten fraction III.

Transmission of antibodies from mother to foetus has been demonstrated in a variety of animals. In man, the route of transmission appears to be across the placenta (Dancis *et al.*, 1961), and the same is true in other primates (Bangham *et al.*, 1958), whereas in rodents the yolk-sac is the important route (Brambell, 1958).

Published observations suggest that this transfer of antibodies across the placenta is selective. Thus Arnon *et al.* (1959) have shown that antibodies to *Escherichia*

coli and *Shigella sonnei* are poorly transmitted, whereas the transfer of diphtheria antitoxin takes place with ease. Our own observations show that comparable differences occur with antibodies to different cow's milk proteins and gluten fraction III. The reason for such differences is not clear, and such factors as molecular size or valency of antibody do not appear to be decisive (Hemmings and Brambell, 1961; Vahlquist, 1958).

Antibodies to most antigens are present in the blood of the offspring at the same or a lower concentration than in the maternal blood (Vahlquist, 1958). However, with some antibodies, such as those to streptolysin-O, the titre is higher in cord blood than in maternal blood (Murray and Calman, 1953; Stück *et al.*, 1957). The present study has shown that antibodies to milk proteins and gluten fraction III are often present at higher titre in cord blood than in maternal blood, especially in the case of antibodies to β -lactoglobulin and fraction III.

Such higher levels of antibody in cord blood could be due to one or more of the following possibilities. First, there may be selective concentration of antibodies by the placenta. Secondly, there may be active formation of antibodies by the foetus *in utero* in response to antigen transmitted from the mother. Thirdly, it is conceivable that a fall in the titre of antibodies in the maternal circulation occurs shortly before or after delivery, but this seems unlikely because the results with maternal sera appear to match those we have obtained with non-pregnant healthy subjects.

As between the first two possibilities—namely, transplacental concentration of antibodies or placental transfer of antigen with foetal immunological competence—our data do not permit of any conclusion. Ratner *et al.* (1953) believe that the foetus may be sensitized *in utero* either actively or passively when pregnant women consume large amounts of highly antigenic foods, but their evidence appears inconclusive.

Whether the titres in cord blood of antibodies to various dietary proteins provide any index of predisposition to disease in infancy or later life is problematical. If such antibodies represent an active immunological response by the infant, then it is conceivable that they might be a manifestation of a state of hypersensitivity to common dietary proteins, and this might be of some importance in later life. If, on the other hand, these foetal antibodies have been acquired passively from the mother, they may exert a protective effect. It is well known that passively transmitted circulating antibodies interfere with active immunization during early infancy (Christie and Peterson, 1951; Osborn *et al.*, 1952; Uhr and Baumann, 1961). It is possible that passively transmitted antibodies to dietary proteins may similarly modify or prevent sensitization of the infant at the time of its first contact with these proteins after birth.

Summary

The levels of circulating antibodies to cow's milk proteins and proteolysed wheat gluten (fraction III) have been determined in the cord blood of 100 newly delivered infants and in the blood of their mothers.

The conspicuous finding was the lack of close correlation between the titres of maternal and cord blood.

In the case of β -lactoglobulin and fraction III it was usual to find higher titres of circulating antibody in cord blood than in maternal blood.

The source of the antibodies in cord blood and their possible influence on subsequent development of hypersensitivity to dietary proteins are discussed.

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REFERENCES

- Acheson, E. D., and Truelove, S. C. (1961). *Brit. med. J.*, **2**, 929.
 Andresen, A. F. R. (1942). *Amer. J. dig. Dis.*, **9**, 91.
 Arnon, H., Salzberger, M., and Olitzki, A. L. (1959). *Pediatrics*, **23**, 86.
 Bangham, D. R., Hobbs, K. R., and Terry, R. J. (1958). *Lancet*, **2**, 351.
 Berger, E., and Bauer, R. (1959). *Med. exp. (Basel)*, **1**, 37.
 Boyden, S. V. (1951). *J. exp. Med.*, **93**, 107.
 Brambell, F. W. R. (1958). *Biol. Rev.*, **33**, 488.
 Christie, A., and Peterson, J. C. (1951). *Amer. J. Dis. Child.*, **81**, 501.
 Dancis, J., Lind, J., Oratz, M., Smolens, J., and Vara, P. (1961). *Amer. J. Obstet. Gynec.*, **82**, 167.
 Davidson, L. (1961). *Brit. med. J.*, **2**, 1358.
 Frazer, A. C., Fletcher, R. F., Ross, C. A. C., Shaw, B., Sammons, H. G., and Schneider, R. (1959). *Lancet*, **2**, 252.
 Gray, J. G. (1961). *Brit. med. J.*, **2**, 1265.
 Gunther, M., Aschaffenburg, R., Matthews, R. H., Parish, W. E., and Coombs, R. R. A. (1960). *Immunology*, **3**, 296.
 Hemmings, W. A., and Brambell, F. W. R. (1961). *Brit. med. Bull.*, **17**, 96.
 Murray, J., and Calman, R. M. (1953). *Brit. med. J.*, **1**, 13.
 Osborn, J. J., Dancis, J., and Julia, J. F. (1952). *Pediatrics*, **10**, 328.
 Ratner, B., Crawford, L. V., and Flynn, J. G. (1956). *Amer. J. Dis. Child.*, **91**, 593.
 Rider, J. A., Moeller, H. C., Devereaux, R. G., and Wright, R. R. (1960). *Acta allerg. (Kbh.)*, **15**, Suppl. 7, p. 486.
 Rowe, A. H. (1942). *Ann. intern. Med.*, **17**, 83.
 Stück, B., Natzschka, J., and Wiesener, H. (1957). *Klin. Wschr.*, **35**, 924.
 Taylor, K. B., Thomson, D. L., Truelove, S. C., and Wright, R. (1961). *Brit. med. J.*, **2**, 1727.
 — and Truelove, S. C. (1961). *Ibid.*, **2**, 924.
 Truelove, S. C. (1961). *Ibid.*, **1**, 154.
 Uhr, J. W., and Baumann, J. B. (1961). *J. exp. Med.*, **113**, 935.
 Vahlquist, B. (1958). *Advanc. Pediat.*, **10**, 305.
 Witebsky, E., and Rose, N. R. (1956). *J. Immunol.*, **76**, 408.

"In general, however, looking at [the food of] other cultures, it is the restriction of choice that is more striking, particularly when one fails to find the food one is used to oneself. Stefansson's Eskimos resented having to eat berries and vegetables, and only did so in times of dire necessity, before eating their dogs, the stage that immediately preceded cannibalism. Other cultures have even stronger taboos. Beef is not appreciated in India, nor is pork in the Arab countries, nor is horse in the English-speaking countries. Preference, habit and taboo may indeed extend the realm of hunger. During the last war, in starving Malta, people threw away powdered eggs in the street rather than eat them. Many other examples could be cited. For most people, it appears, food to be eaten must be good food. Mere nutritiousness is not enough. But in the peculiar conquest of hunger now proceeding in the well-fed countries among those intent on slimming—in the interests of fashion perhaps rather than of health—a new consciousness of nutritive values is growing up and is already affecting taste. There is a sort of perverse injustice about the same world having two kinds of slim people, one kind because they eat only the titbits or imitate the primitive belt-tightening by alternate overeating and starving, the other because their food is inadequate." (*World Health*, September–October, 1962. Special issue on food.)