Z. Ernährungswiss. 20, 283–290 (1981)

ISSN 0044-264 X

National Research Centre), Faculty of Medicine, Al-Azhar University**) and University College for Women, Ain Shams University, Cairo***)*

Biochemical studies on the effect of breast and artificial feeding in newborn Egyptian infants I. Serum proteins and immunoglobulins in 1–4-day-old newborns

M. F. S. El-Hawary), A. A. Soliman**), S. A. Nosseir**),* and *T. S. Kholief***)*

(Received August 21, 1981)

Milk is a naturally balanced diet, which meets the needs of the newly borns, its value has been studied by many workers, as early as (1922) Lewis and Wells reported a rise in euglobulins in colostrum-fed infants compared with artificially-fed ones. The difference in incidence of enteric infections in breast-fed and in bottle-fed infants is well known. It is hard to judge whether this difference is due to a beneficial effect of breast milk or an increased contamination during bottle feeding. Tassovatz and Kotsitch (1961) reported that administration of unprocessed breast milk ceased an epidemic of enterocolitis caused by E. Coli 0 111 B_4 which remained uncontrolled for 5 months in infants inspite of the use of classical epidemiological measures. Crose (1966) had never seen an enteropathogenic E. Coli infection in a breast-fed newborn. Matta and Uruttia (1971) showed that diarrhea was uncommon in breast fed infants though exposure to Shigella, enteropathogenic E. Coli and Salmonella was common. Breast milk feeding protects against respiratory infections (Mellander et al., 1959). Therefore, it has been planned to carry out follow up comparative studies for the quantitative estimation of serum proteins and immunoglobulins in different groups of newborn infants at different ages as well as their mothers. The present study deals with such investigations on newborn infants during the period of colostrum feeding as compared to newborns who from the first hour of their life recieve artificial feeding.

Material and methods

We studied 31 infants of 1–4 days old: 16 were fed breast milk and 15 were fed artificial milk. All cases studied had gestation periods greater than 266 days, cases with mothers having anaemia, infection, hypertension, preeclampsia or rhesus disease were excluded. All infants weighed 2500 g or more at birth. Thorough clinical and laboratory investigations including blood picture, urine and stool analysis proved the studied cases to be normal. The lactating mothers of these infants have been included in this study. 17 normal non-lactating females have been also included to serve as controls for the lactating mothers.

Blood samples were collected from venous blood from the antecubital vein or the external Jugular vein for the mothers and infants, respectively. Serum was separated and kept at -20°C till analysis. Total protein estimation was performed by the biuret method as described by Wootton in 1973. Electrophoretic fractionation of proteins has been done by simple agar gel electrophoresis as described by El-Hawary and Ebrahim (1968). The immunoelectrophoretic pattern was performed using polyvalent antihuman serum prepared in rabbits (Hirshfeld, 1960). The double immunodiffusion was carried out as described by Nossier (1974). The specific antisera and the specific immunoplates for I_gG, I_gA, I_gM were obtained from Behringwerke Laboratories (Germany).

Results

The data obtained for total serum proteins and its fractions for the different groups studied are shown in table 1 and the data for their serum immunoglobulins (I_gG, I_gM, I_gA) are given in tables 2–5.

Discussion

Total serum protein level in infants fed on colostrum was found to exceed the level encountered in artificially-fed infants. It almost matched the level in their mothers but a little lower than the mean found for normal non-lactating control females. This agrees with similar reports by Miller and Weller (1971). Also, of interest is that the mean albumin level in

Table 1. Serum total proteins and their main fractions (g%) in the different groups included in the study.

	TSP	Alb	α_1-g	α_2-g	β-g	γ-g	TG	Alb/TG
Normal non-lactating control females (17 cases aged 23–40 years)								
Range	5.86–	2.87–	0.1–	0.44–	0.62–	1.25–	2.71	0.86–
	7.45	4.27	0.33	0.80	1.04	1.81	3.45	1.42
Mean	6.62	3.56	0.24	0.56	0.68	1.50	3.06	1.16
± S.E.	0.10	0.06	0.01	0.02	0.03	0.02	0.06	0.04
Totally colostrum-fed infants (16 cases aged 1–4 days)								
Range	5.82–	2.90–	0.24–	0.59–	0.59–	1.21–	2.86–	0.89–
	6.95	3.79	0.39	0.79	0.87	1.54	3.24	1.19
Mean	6.25	3.19	0.28	0.69	0.72	1.36	3.06	1.04
± S.E.	0.17	0.15	0.02	0.03	0.04	0.05	0.06	0.05
Totally artificially fed infants (15 cases aged 1–4 days)								
Range	5.64–	2.79–	0.18–	0.60–	0.56–	1.06–	2.65–	0.96–
	5.79	3.01	0.33	0.78	0.86	1.22	2.95	1.12
Mean	5.72	2.88	0.25	0.68	0.74	1.15	2.84	1.01
± S.E.	0.03	0.05	0.03	0.04	0.05	0.03	0.06	0.03
Mothers of colostrum-fed infants (16 cases aged 17–35 years)								
Range	5.99–	2.60–	0.20–	0.64–	0.91–	1.30–	3.29–	0.72–
	6.75	2.88	0.58	0.85	1.05	1.78	4.02	0.82
Mean	6.39	2.74	0.40	0.72	1.11	1.53	3.64	0.75
± S.E.	0.12	0.04	0.06	0.03	0.07	0.08	0.12	0.02

TSP: total serum proteins, Alb: albumin, α_1-g: alpha$_1$ globulin, α_2-g: alpha$_2$ globulin, β-g: beta globulin, γ-g: gamma globulin, TG: Total globulins.

Table 2. Qualitative and quantitative levels of immunoglobulins I_gG, I_gM, I_gA for 17 non-lactating control females.

Immunoelectrophoresis using						Immunodiffusion					
Polyvalent antihuman sera			Monospecific antisera			Double			Radial		
I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA
									in mg/100 ml serum		
N	N	N	N	N	N	N	N	N	1250	95	290
N	N	N	N	N	N	N	N	N	1530	160	230
N	N	N	N	N	N	N	N	N	1500	120	200
N	N	N	N	N	N	N	N	N	1200	150	250
N	N	N	N	N	N	N	N	N	1400	126	150
N	N	N	N	N	N	N	N	N	1095	126	140
N	N	N	N	N	N	N	N	N	1040	126	175
N	N	N	N	N	N	N	N	N	1060	70	165
N	N	N	N	N	N	N	N	N	1520	126	280
N	N	N	N	N	N	N	N	N	1250	126	165
N	N	N	N	N	N	N	N	N	1170	95	230
N	N	N	N	N	N	N	N	N	1250	150	290
N	N	N	N	N	N	N	N	N	1400	180	230
N	N	N	N	N	N	N	N	N	1300	165	160
N	N	N	N	N	N	N	N	N	1090	126	230
N	N	N	N	N	N	N	N	N	1080	70	190
N	N	N	N	N	N	N	N	N	1390	180	160
Range									1040–1530	70–180	140–290
Mean ± S.E.									1265 33.13	128 8.08	207 12.15

N: normal.

colostrum fed infants is higher than in artificially-fed ones. Such findings, although could obviously indicate the importance of colostrum as nutritive material for the newborn infants, yet the cause for this difference in albumin level is unfortunately not very clear. One might suggest however, that colostrum itself might play a very important contributing role in the facilitation of absorption of macromolecular substances including albumin (Hardy, 1969). An alternative suggestion could be the proper and better synthesis of albumin by the liver as helped through the supply of colostrum that contains very high class proteins. Thus, in contrast with artificial milk most of which contains about 1.5–3 g% proteins while natural colostrum is very rich in proteins and may contain up to 8.5 g% proteins (El-Diwany and Mokhtar, 1966; Wright, 1971).

Alpha and beta globulins exhibit almost equal levels in both colostrum- and artificially-fed infants. It seems that colostrum feeding at this very early age has no differential influence on these globulins. The significance of these results is not obvious but one might add that since alpha and beta globulins have only a slight implication in the immunological or nutritive

Table 3. Qualitative and quantitative levels of immunoglobulins I_gG, I_gM, I_gA for 16 totally colostrum-fed infants.

	Immunoelectrophoresis using						Immunodiffusion					
	Polyvalent antisera			Monospecific antisera			Double			Radial		
S.No.	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA
										in mg/100 ml serum		
1	N	–	–	N	T	–	N	T	–	1220	8	–
2	N	–	–	N	–	T	N	–	T	1350	–	4
3	N	–	–	N	–	T	N	–	T	1300	–	2
4	N	–	–	N	–	T	N	–	T	1200	–	6
5	N	–	–	N	–	–	N	–	–	1300	–	–
6	N	–	–	N	T	T	N	T	T	1400	7	3
7	N	–	–	N	–	–	N	–	–	1300	–	–
8	N	–	–	N	–	T	N	–	T	1285	–	4
9	N	–	–	N	T	–	N	T	–	1310	7	–
10	N	–	–	N	–	T	N	–	T	1295	–	4
11	N	–	–	N	T	T	N	T	T	1290	2	5
12	N	–	–	N	T	–	N	T	–	1275	8	–
13	N	–	–	N	–	T	N	–	T	1295	–	6
14	N	–	–	N	T	T	N	T	T	1280	8	4
15	N	–	–	N	–	–	N	–	–	1305	–	–
16	N	–	–	N	–	T	N	–	T	1315	–	2
Range										1200–1400	0–8	0–6
Mean ± S.E.										1295 ± 30.95	2.5 ± 1.30	2.5 ± 0.71

T: Traces.

processes. There is a possibility that the newborn infant protein machinary is busy in forming antibodies necessary, protein components, namely albumin, the immunoglobulins necessary for his well being in the new extrauterine enviroment. The demonstration of higher values for serum gamma globulins in the colstrum fed group as compared to the artificially-fed infants, yet, the facilitating effect exerted by colostrum helping maximum intestinal absorption of its higher protein content cannot be ruled out. Furthermore, the rise in serum gamma globulins in colostrum-fed infants may be due to active antigenic stimulation of its protein content. The dependence of serum gamma globulins on intestinal antigenic stimulation has been established by Wostmann and Gordon (1960), Sterl et al. (1960) and Sacquet and Vargue (1961). Moreover, breast feeding has been found to promote normal bacterial colonization of gastrointestinal tract of mammals including man, Smith and Crab (1966).

Serum I_g level in colostrum-fed infants was 1295 mg %, being significantly higher than in artificially-fed ones, showing a mean of 1020 mg %. It is established that most of the newborn infants, serum I_g is derived from his mother through placental transfer, particularly during the last trimes-

Table 4. Qualitative and quantitative levels of immunoglobulins I_gG, I_gM, I_gA for 15 totally artificially fed infants.

	Immunoelectrophoresis using						Immunodiffusion					
	Polyvalent antisera			Monospecific antisera			Double			Radial		
S.No.	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA
										in mg/100 ml serum		
1	N	–	–	N	–	T	N	–	T	1100	–	2
2	N	–	–	N	–	–	N	–	–	1000	–	–
3	N	–	–	N	T	–	N	T	–	1150	8	–
4	N	–	–	N	–	–	N	–	–	900	–	–
5	N	–	–	N	T	–	N	T	–	950	4	–
6	N	–	–	N	–	–	N	–	–	1000	–	–
7	N	–	–	N	–	–	N	–	T	1020	–	2
8	N	–	–	N	T	–	N	T	–	1010	8	–
9	N	–	–	N	–	–	N	–	–	1040	–	–
10	N	–	–	N	–	T	N	–	T	1020	–	2
11	N	–	–	N	T	–	N	T	–	1030	5	–
12	N	–	–	N	T	–	N	T	–	1015	5	–
13	N	–	–	N	–	–	N	–	–	1035	–	–
14	N	–	–	N	T	–	N	T	–	1025	6	–
15	N	–	–	N	–	–	N	–	–	1005	–	–
Range										9900–1150	0–8	0–2
Mean ± S.E.										1020 45.40	0.4 1.19	2.4 0.10

T: Traces.

ter of intrauterine life (Kohler and Farr, 1966) as well as during delivery (Yang et al., 1971) and Cochran (1972). At the same time, there is much controversy regarding the newborn capability to synthesize his own I_gG. If all newborn serum I_gG at this age is derived through placenta rather than partial endogenous synthesis, then one may not find significant difference in its level in colostrum- and artificially fed infants. However, our data indicate that there are certain factors that influence the serum I_gG among the newborns, among which one must mention the contribution of fresh colostrum I_gG passing through the intestinal wall and remaining in a biologically-active form (Pierce et al., 1964).

Serum I_gM in the tested cases was detected in 12 out of the 31 cases with almost equal distribution between colostrum- and artificially fed infants. IgM has a high molecular weight and is unable to cross the placental barrier (von Muralt, 1963, and Adinolfi and Humphrey, 1969). The small amount of I_gM detected in our cases may be due to its synthesis by foetus or the newborn at a very low rate (Smith, 1960, and West et al., 1962, and El-Hawary et al., 1974). This might be coincident with the development of intestinal flora in these infants because I_gM synthesis has been shown to occur after short latent period after introduction of the antigen (Uhr and Finkelstein, 1971).

Table 5. Qualitative and quantitative levels of immunoglobulins I_gG, I_gM, I_gA for 16 mothers of colostrum-fed infants.

	Immunoelectrophoresis using						Immunodiffusion					
	Polyvalent antisera sera			Monospecific antisera			Double			Radial		
S.No.	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA	I_gG	I_gM	I_gA
										in mg/100 ml serum		
1	N	I	D	N	I	D	N	I	D	1000	250	98
2	N	N	N	N	N	N	N	N	N	1300	126	350
3	N	I	N	N	I	N	N	I	N	1320	250	290
4	N	I	N	N	I	N	N	I	N	1250	260	380
5	N	N	N	N	N	N	N	N	N	1000	100	160
6	N	I	N	N	I	N	N	I	N	1200	246	150
7	N	N	N	N	N	N	N	N	N	1168	195	228
8	N	N	N	N	N	N	N	N	N	1178	200	238
9	N	N	D	N	N	D	N	N	D	1179	190	200
10	N	I	N	N	I	N	N	I	N	1188	205	230
11	N	N	D	N	N	D	N	N	D	1158	180	220
12	N	I	N	N	I	N	N	I	N	1188	215	276
13	N	I	N	N	I	N	N	I	N	1160	220	238
14	N	I	N	N	I	N	N	I	N	1198	210	256
15	N	I	N	N	I	N	N	I	N	1196	205	246
16	N	I	N	N	I	N	N	I	N	1168	230–	248
Range										1000–1320	100–260	98–380
Mean ± S.E.										1178 ± 58.90	205 ± 29.45	238 ± 43.72

The serial number in table 3 corresponds to the serial number in this table.

A significant difference has been encountered; serum I_gA was detected in 63 % of colostrum-fed infants while it was detected in 20 % only of artificially fed ones. Stiehm and Fubenberg (1966) detected it in one third of his samples of cord blood. The high percentage incidence as well as high serum concentration of serum I_gA among infants receiving colostrum could be due to: (a) the high colostrum I_gA content which can pass through the intestinal mucosa to reach the blood circulation, (b) possibly due to increased synthesis due to a possible antigenic stimulant in fresh colostrum (Barret, 1970, and Roitt, 1973).

Serum total proteins of lactating mothers was less than in normal non-lactating females; the decrease appears to affect mainly albumin and gamma globulin. The decrease in serum albumin, seems to be multifactorial, among these factors are: (a) increased demands in absence of adequate intake during pregnancy and lactation (Miller et al., 1971, and Roman, 1971), (b) loss of proteins (in blood during delivery) and transplacental passage to foetus during vaginal delivery (Cochran, 1972), through a process of ultrafiltration owing to its small size and oval shape. Cochran (1972) suggested that uterine contractions during labour might cause

ultrafiltration of proteins which lead to lower albumin level in mothers than infants. The levels of alpha and beta globulins were higher than controls and also than the infants is in agreement with reports by Roman (1971). Lowered serum gamma globulin level in lactating mothers than the controls might be due to the transplacental transfer process (Wootton, 1973), as well as its excretion in colostrum Blackmore and Garner (1965). However, there is no significant difference between serum gamma globulin levels in mothers and their infants in agreement with the observation by Von Muralt (1963).

Summary

Investigations on the effect of colostrum feeding in 1–4-day-old newborn infants on serum proteins and their immunoglobulins were carried out. The values for serum total proteins, albumin and gamma globulins are higher in colostrum-fed infants than the artificially fed group. Immunoglobulin G and immunoglobulin A levels were significantly higher, while immunoglobulin M level was only slightly elevated in the former group.

On the other hand, serum total proteins as well as albumin and gamma globulins levels were decreased in lactating compared with non-lactating females, while the alpha and beta globulins levels were higher for lactating than controls. For individual immunoglobulins; I_gG level was lower and I_gA, I_gM levels were higher for lactating than non-lactating females.

References

1. Lewis, J. H., H. G. Wells: J. Amer. med. Ass. **78,** 863 (1922).
2. Tassovatz, B., A. Kotsitch: Annals de Pédiatrie **8,** 285 (1961).
3. Crose, V. M.: In: The premature baby, p. 143 Churchill (London 1969).
4. Matta, L. J., J. J. Uruttia: Ann. N. Y. Acad. Scie. **176,** 93 (1971).
5. Mellander, O., B. Vahlquist, T. Melbin: Acta Paed., **48,** suppl. 116 (1959).
6. Wootton, I. D. P.: Microanalysis in Medical Biochemistry, 4th edition J. & A. Churchill (editors) p. 138–150 (London 1973).
7. El-Hawary, M. F. S., Ebrahim, A. M.: J. Egypt. Med. Assoc., **51,** 750 (1968).
8. Hirshfeld, J.: Science Tools **7,** 18 (1960).
9. Nosseir, S. A.: M. D. Thesis, Faculty of Medicine Al-Azhar University (1974).
10. Miller, S. E., M. J. Weller: A Text Book of Clinical Pathology, 8th edition p. 244 (1971).
11. Harding, R. N.: J. Physiol. (London) **204,** 607 (1969).
12. El-Diwany, M., N. Mokhtar: Composition of human milk. In: Basic Paediatrics, 3rd edition, p. 89 (1966).
13. Wright, S.: In: Applied physiology, revised by Keels, C. A., and E. Neil. 12th edition, Oxford University Press p. 527 (1971).
14. Wostmann, B. S., H. A. Gordon: J. Immunol., **84,** 27 (1960).
15. Sterzl, J., J. Kostka, I. Rihat, L. Mandel: Folia Microbiol., **5,** 29 (1960).
16. Sacquet, E., R. Vargue: Ann. Inst. Pasteur, **101,** 703 (1961).
17. Smith, H. W., W. E. Crabb: J. Path. Bact., **82,** 53 (1966).
18. Kohler, P., R. Farr: Nature, **210,** 1070 (1966).
19. Yang, S., A. M. Kleinman, E. B. Rosenberg, P. Wei: Amer. J. Obst. Gyn., **109,** 78 (1961).
20. Pierce, A. E., P. C. Risdall, B. Show: Calf J. Phys. **171,** 203 (1964).
21. von Muralt, G.: In: W. H. Hitzig (editor), Die Plasmaproteine in der klinischen Medizin, Springer-Verlag, p. 105 (Berlin 1963).

22. Adinolfi, M., J. Humphrey: Immunology and development Spastics International Medical Publications, p. 38 (London 1969).
23. Smith, R. T.: Response to active immunization of human infants during the neonatal period. In Ciba foundation Symposium on cellular aspects of immunity, G. E. W. Wolsten and M. O. Connor (editors), Brown little, p. 3 (1960).
24. West, C. D., H. Hong, N. H. Holland: J. Clin. Invest., **41**, 2059 (1962).
25. El-Hawary, M. F. S., E. M. El-Bassousy, M. Hafez, A. A. Khashaba, F. A. El-Shobaki, A. M. El-Shazly: Gaz. Egypt. Paed. Assoc., **22**, 67 (1974).
26. Uhr, T. W., M. S. Finkelstein: Prog. Allergy, **83**, 1037 (1967).
27. Stiehm, R. E., H. H. Fubenberg (1966): A Survey. Paediatrics **37**, 715 (1966).
28. Barret, J. T.: Text book of Immunology, An introduction to immunochemistry and immunobiology. The C. V. Mosby Co F. 174 (1970).
29. Roitt, I.: In: Essential Immunology, Blackwell Scientific Publications 5th printing, p. 146 (1973).
30. Miller, J. F. A., G. F. Mitchell, N. S. Weiss: Nature, **214**, 992 (1967).
31. Roman, W.: S. Aust. Clin., **52**, 165 (1971).
32. Blackmore, F., R. J. Garner: J. Comp. Path., **66**, (4), 287 (1965).
33. Cochran, T. E.: J. Obst. Gyn. (British), **97**, 238 (1972).

Authors' address:

Prof. Dr. M. F. S. El-Hawary, Chairman Biochemistry Department,
National Research Centre, Dokki, Cairo (Egypt)