Comparative Analysis of Human Milk and Human Blood Plasma by Means of Diffusion-in-Gel Methods

In the immunological analysis of antigenic substances in biological fluids diffusion-in-gel techniques have been used to an increasing extent during the last years. In particular the double diffusion technique of OUCHTERLONY[1] and the immune electrophoretic method of GRABAR and WILLIAMS[2] have shown to be advantageous in the analysis of protein mixtures, such as human blood plasma. By means of the latter technique at least twenty antigenic factors have been demonstrated in blood plasma, several of which have been identified with known blood serum proteins. The human plasma – anti-human plasma spectrum has been used as a reference in this investigation which concerned antigenic factors in human milk that can be serologically identified with blood serum proteins.

As has been shown by GUGLER *et al.*[3] and HANSON and JOHANSSON[4–6] a large number of antigenic factors can be demonstrated in human milk with diffusion-in-gel techniques using homologous immune sera and anti-human plasma sera. Since these factors seemed to vary quantitatively and qualitatively at different times during the lactation period precolostrum taken about three weeks before parturition, colostrum taken within one day of parturition and mature milk taken three weeks after parturition were used in this study.

Immune electrophorectic analysis of, for example, colostrum with homologous rabbit immune sera showed a spectrum consisting of fourteen separate immune precipitates (Fig. 1a). These were localized throughout the regions of albumin to γ-globulin when compared with agar electrophoresis of human plasma. Several of the antigenic factors in human milk were related to antigenic factors in plasma since colostrum analyzed with anti-human plasma serum showed a spectrum of twelve separate precipitation lines. This relationship was also demonstrated when human plasma was analyzed with anti-colostrum serum as a precipitation spectrum of ten lines was then formed. In order to identify these antigenic factors in milk, preparations of isolated blood serum proteins and immune sera against purified blood serum proteins were used. Some examples of these identification experiments will be given.

With the double diffusion technique (WADSWORTH[7]) two separate precipitation lines could be shown in precolostrum, colostrum, and mature milk using an immune serum against prealbumin rich in tryptophane (Fig. 1b). In immune electrophoretic analysis of milk and blood plasma with this anti-prealbumin serum two precipitates were formed – one in the electrophoretic region of prealbumin and another in the α_1-globulin region.

Using preparations of respectively acid seromucoid and α_1-glucoprotein (3·5 S), these two blood serum proteins were identified in precolostrum, colostrum, and mature milk as seen in Fig. 1c. The experiments were performed according to WADSWORTH and HANSON[8]. An extra basin for the reference protein preparation was made parallel

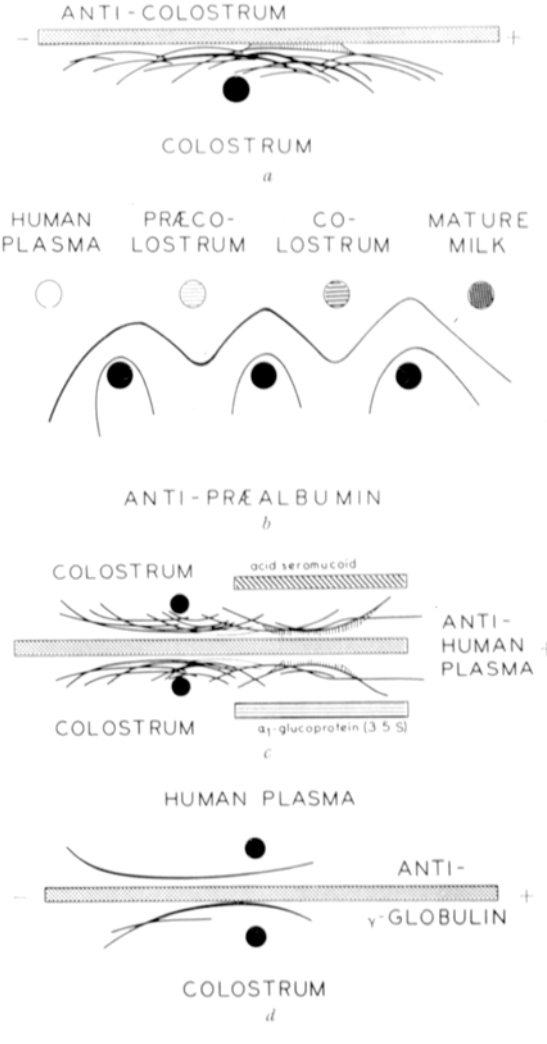

Fig. 1

(a) Diagram of immune electrophoretic analysis of colostrum by means of anti-colostrum serum

(b) Diagram of comparative analysis of human blood plasma, precolostrum, colostrum, and mature milk by means of anti-prealbumin serum

(c) Diagram of identification of the precipitates corresponding respectively to acid seromucoid and α_1-glucoprotein (3·5 S) in the immune electrophoretic spectra of colostrum-anti-human plasma

(d) Diagram of the immune electrophoretic analysis of human blood plasma and colostrum by means of anti-γ-globulin serum

to the immune serum basin in the immune electrophoretic analysis of colostrum. A precipitate in the colostrum-anti-human plasma spectrum identified with the known precipitate formed by the protein preparation and the immune serum.

Transferrin was found to exist in milk from different parts of the lactation period as an immune serum against transferrin gave one precipitation line with milk using double diffusion technique. This precipitate corresponded

[1] Ö. OUCHTERLONY, *Progr. Allergy*, vol. 5 (Karger, Basel/New York 1958), p. 1.

[2] P. GRABAR et C. A. WILLIAMS, Biochim. biophys. Acta *17*, 67 (1955); P. GRABAR, *Advanc. Protein Chem.*, vol. 13 (Academic Press New York 1958), p. 1.

[3] E. GUGLER, G. BOKELMANN, A. DÄTWYLER, and G. VON MURALT, Schweiz. med. Wschr. *88*, 1264 (1958).

[4] B. JOHANSSON, Nature *181*, 996 (1958).

[5] L. Å. HANSON and B. JOHANSSON, Int. Arch. Allergy *15*, 260 (1959).

[6] L. Å. HANSON, Int. Arch. Allergy *15*, 248 (1959).

[7] C. WADSWORTH, Int. Arch. Allergy *10*, 355 (1957).

[8] C. WADSWORTH and L. Å. HANSON (in Manuscript).

to transferrin as was shown in immune electrophoretic experiments and in experiments where a pure preparation of transferrin was used as control.

Finally the proteins in human milk that were serologically related to blood serum γ-globulin should be mentioned. Immune electrophoretic analysis of milk by means of an anti-γ-globulin serum showed two precipitation lines. One of these was mainly localized in the area corresponding to the β_2-globulin fraction of blood serum. The other one crossed over the first and had its maximum somewhat further out in the γ-globulin region (Fig. 1d). Analysis of milk by means of the anti-γ-globulin serum using the double diffusion technique gave results which indicated that there may be more than these two precipitates. Analysis of an immunologically pure γ-globulin preparation by means of anti-milk sera showed corresponding results.

Serological Identification of Blood Plasma Proteins in Human Milk

Human Plasma	Pre-colostrum	Colostrum	Mature Milk
Prealbumin	+	+	+
Albumin	+	+	+
Acid Seromucoid	+	+	+
α_1-gluco-protein (3·5 S)	+	+	+
Ceruloplasmin	+	+	(+)
Haptoglobin	—	+	+
α_2-or β-lipoprotein	+	+	(+)
Transferrin	+	+	+
Fibrinogen	(+)	(+)	—
β_{2A}-globulin	+	+	+
β_{2M}-globulin	+	+	+
γ-globulin	+	+	+

The Table shows the blood plasma proteins that can be serologically identified in human milk. Prealbumin, acid seromucoid, α_1-glucoprotein (3·5 S), transferrin, and γ-globulin have already been mentioned. Also albumin, ceruloplasmin, haptoglobin, α_2-or β-lipoprotein, fibrinogen, β_{2A}-globulin, and β_{2M}-globulin have been found. Owing to the great sensitivity of the techniques used very small amounts of antigenic material are demonstrable and it should be noted that these proteins probably make up only a small part of the protein content of human milk.

The experiments will be reported in detail elsewhere.

The purified blood serum proteins were kindly supplied by Behring Werke, Marburg, Germany and by Docents I. BRATTSTEIN and J. GOA, Dept. of Medical Chemistry, University of Gothenburg.

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Zusammenfassung

Mit Hilfe von Immunoelektrophorese und Doppeldiffusiontechnik wurden in Humanmilch an Blutplasmaproteinen serologisch identifiziert: Praealbumin, Albumin, Acid Seromucoid, α_1-Glykoprotein (3·5 S), Coeruloplasmin, Haptoglobin, α_2- oder β-Lipoprotein, Transferrin, Fibrinogen, β_{2A}-Globulin, β_{2M}-Globulin und γ-Globulin.

Embryonalentwicklung der Termite *Kalotermes flavicollis*

Die in ganz Südeuropa beheimatete Termitenart *Kalotermes flavicollis* zeigt, wie viele Termitenarten, eine ausserordentlich langsame Embryonalentwicklung. Diese dauert durchschnittlich 54 Tage bei 26°C. Durch Eintauchen in Paraffinöl können die Eier infolge Aufhebung von Brechungserscheinungen durchsichtig gemacht werden. So war es auch möglich, die Entstehung und die Umlagerungen des Termitenkeimes in einem Zeitrafferfilm festzuhalten.

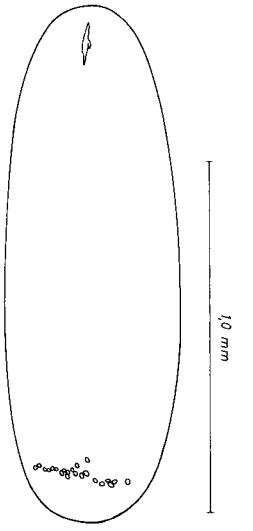

Abb. 1. Dorsalansicht des Eies von *Kalotermes flavicollis*
Unten: Hinterpol mit zahlreichen Mikropylen.
Oben: Strukturfreies, fensterartiges Feld am Vorderpol, das möglicherweise beim Gasaustausch eine Rolle spielt und gleichzeitig die Stelle markiert, wo die Larve später schlüpft.

Im Zusammenhang mit Dotterbewegungen gelangen die Furchungskerne an die Oberfläche des Eies und bilden dort bis zum 6. Tag der Entwicklung das Blastoderm und die Keimanlage aus. Diese entsteht direkt unter den hufeisenartig angeordneten 14–26 Mikropylen (Abb. 1) auf der dorsalen Seite des Hinterpols zwischen 0 und 13% der Eilänge. Das Amnion faltet sich, im Gegensatz zu den meisten übrigen hemimetabolen Insekten, ohne jegliche Einrollung an der Peripherie des Dotters durch Umklappen des hinteren Randes der Keimscheibe, wodurch eine kugelförmige Keimblase entsteht, die im optischen Schnitt als Ringfrom erscheint. Sie verschiebt sich in der Folgezeit auf die ventrale Seite und wächst dort zum fertig segmentierten Keimstreif aus, dessen hinteres Ende eine Kaudalkrümmung der letzten drei Abdominalsegmente zeigt. Dadurch kommt eine schwache Einsenkung des Keimes in den Dotter zustande.

Mit dem 31. Tag beginnen die eigentlichen Bewegungen des Keimes, deren erste Phase der «Ausrollung» beim invaginierten Typ entspricht. Der Kopfpol gleitet um das Hinterende herum auf die Dorsalseite und nach vorne, wobei er schliesslich die Zellen der Serosa und des Amnions am Vorderteil des Eies zum sogenannten Dorsalorgan zusammenschiebt.

Die zweite Phase der Blastokinese wird durch starke Kontraktionen des Dotters am 32. Tage eingeleitet. Dies ist der Auftakt zu einer Rotation des Keimes um 180°, die sich am 34. Tag vollzieht, und zwar um den Dotter herum, wobei der Drehpunkt mit der Eilängsachse zu-