

COMPARATIVE ELECTROPHORETIC STUDIES OF BOVINE AND HUMAN
COLOSTRUM IN RELATION TO NEO-NATAL IMMUNITY.

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Schneider and Szathmary (1939) have shown that the transmission of passive immunity to the foetus *in utero* and hence to the newborn at the time of birth is dependent upon the histological structure of the placenta. On the basis of the placental structure they divide the animals studied, either by themselves or by other workers, into four main groups. Group I with a placenta epithelio-chorialis is characterised by the absence of intra-uterine transfer of immunity. Any immunity of the newborn is dependent upon the ingestion of specific antibodies which are absorbed as unchanged globulins after the first suckle. This group includes the pig, horse, bovine and goat. At the other end of the scale is Group IV with a placenta haemochorialis. The immunity of the newborn of this group including man, rabbits, guinea-pigs, mice and rats results from intra-uterine transfer of specific antibodies. Apparently this immunity is not augmented by the ingestion and absorption of specific antibodies from the colostrum milk.

Alexander and Mason (1941) in a study of transmitted immunity to horsesickness in foals showed by an investigation of the antibody content of the serum of foals out of immune dams that at birth no antibodies could be detected in the serum of foals. Within 30 hours of the first ingestion of colostrum antibodies were not merely detectable but had reached a concentration not lower than that in the serum of the dam. This passive immunity afforded complete protection to the injection of fully virulent virus. It was assumed that the source of these antibodies was the colostrum milk.

Jameson and his co-workers (1942) in an electrophoretic study of calf sera demonstrated the absence of γ globulins in the serum of calves at birth and their appearance in high concentration during the early nursing period.

Polson (1943) in a preliminary communication showed electrophoretically the absence of γ globulins and only traces of β globulins in the serum of foals at birth. At the age of 5 days the β globulins had increased tremendously in concentration and the γ globulins, representing the specific antibodies, were easily demonstrable.

Subsequently in an unpublished experiment (Alexander and Polson) these observations were confirmed in foals out of immune and susceptible dams. In addition it was shown that a foal out of an immune dam, that was fed colostrum from a susceptible dam as its first meal and then returned to be nursed by its own dam, was fully susceptible to horsesickness in that no antibodies were detectable in the serum and it succumbed to horsesickness as a result of infection with

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virulent blood. On the other hand a foal out of a susceptible dam contained no humoral antibodies at birth. Antibodies appeared after the first feed of colostrum from an immune dam and the foal was resistant to artificial infection while being nursed by its susceptible dam.

Later Smith (1948) confirmed the part played by the colostrum in the production of passive immunity in calves. From his study of the lactoglobulins of the colostrum and the γ globulins of the serum he concluded that there was a difference in electrophoretic mobility which justified the conclusion that the two are not identical. This finding is not in agreement with the conclusions drawn from the present series of experiments and is discussed below.

All the above work has been done on animals which belong to Schneider and Szathmary's group I. As a result of active collaboration by a practising gynecologist and by members of the staff of the Moedersbond Maternity Hospital and the Pretoria General Hospital, to whom I take this opportunity of expressing my very sincere thanks, some data have been collected from man as a representative of Schneider's Group IV. These results, together with further experimental data on the transmission of passive immunity to calves, form the basis of this communication.

Method.

The electrophoresis apparatus used in this study was that described by Polson (1945) in conjunction with the Lamm scale method of registering the moving boundaries.

To obviate the possibility of denaturing globulins in the milk and colostrum the casein was precipitated by the enzymatic action of rennet, using the Standard Cheese Rennet manufactured by Chr. Hansen's Laboratory Limited, Reading, Berks., England.

The electrophoresis experiments were carried out in buffer at pH 8.6 and the mobilities were calculated from the descending column.

The bovine sera were obtained from animals in the Institute's dairy herd. The human sera were collected from the umbilical cord. It is regretted that for the sake of completeness it was not possible to obtain serum samples from infants at various periods after feeding.

Results.

The results are presented in the series of electrophoretic diagrams in Chart I which should be read in conjunction with the mobilities of the main components in bovine serum and colostrum, and human serum and colostrum in Tables 1 and 2 respectively. For the sake of ready reference the electrophoretic diagram of the serum from a non-pregnant woman is shown in figure 5 of the chart.

It is evident that there is a fairly close similarity between the main components of bovine and human serum except that the human pregnant serum at birth contains a well defined component with a mobility possibly slightly less than the β globulin. The identity of this component is not known. On the other hand there are very marked differences between bovine and human colostrum and between calf and infant serum at birth.

TABLE 1.—*Mobilities of Main Components in Bovine Serum and Colostrum.*

Material.	Albumin.	Mobilities $\times 10^5$ cm./volt./sec.			
		α Glob.	β Glob.	α Glob.	
				1.	2.
Maternal Serum.....	6.68	6.85	4.34	2.05	0.91
Calf's Serum before Suckling.....	8.63	6.70	4.06	—	—
Colostrum.....	—	6.85	4.4	1.94	± 1.14
Calf's Serum after Suckling.....	8.63	6.6	3.95	2.1	1.17

TABLE 2.—*Mobilities of Main Components in Human Serum and Colostrum.*

Material.	Albumin.	Mobilities $\times 10^5$ cm./volt./ sec.		
		α Glob.	β Glob.	α Glob.
				—
Maternal Serum at Birth of Baby.....	8.23	5.9	4.11	1.37
Baby's Serum at Birth.....	8.00	6.01	4.11	1.60
Colostrum.....	—	5.48	3.75	—

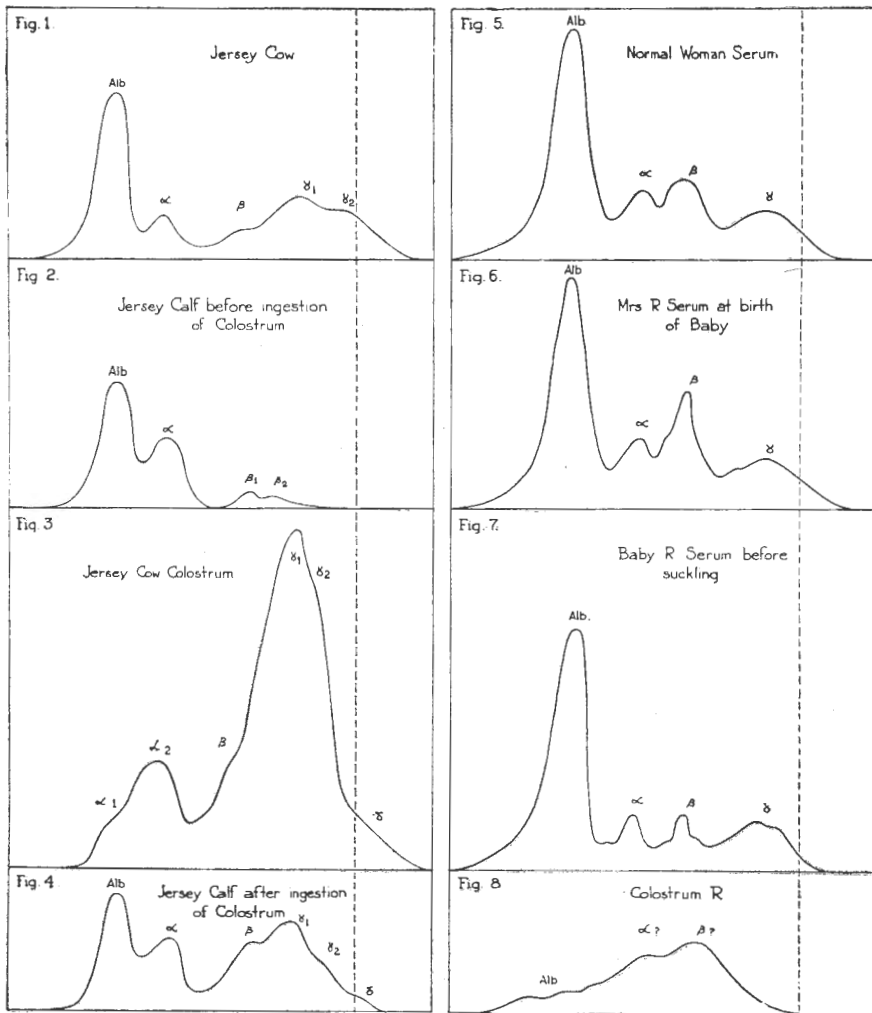
In bovine colostrum the albumin fraction is conspicuous by its absence whereas it is present, though in very low concentration, in the human. In the bovine colostrum the predominant feature is the exceedingly high concentration of the α globulins (γ_1 and γ_2) whereas these in turn are conspicuous by their absence from human colostrum.

The calf at the time of birth has no γ globulins in the serum though they are present in high concentration within a few hours of the first feed of colostrum. The infant at birth shows the presence of γ globulins in a concentration remarkably similar to that of its mother.

In considering the calculated mobility rates of the various components it is seen from Table I that, in the bovine series, there are no significant differences except in the β globulin fraction where the mobility of this component in the calf's serum is lower. (The difference in mobility of the α globulin is not significant.) When considering the human series in Table 2 the calculated figures for both the β and α globulins in the colostrum appear to be lower than in the maternal or infant blood serum. In both series slight differences are noted in the mobilities of the γ globulins.

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CHART 1.



Discussion.

The opinion is expressed that the minor differences in calculated mobility rates of the various components noted above are more apparent than real. When considered in conjunction with the diagrams it will be seen that differences in calculated mobilities occur when two or more components are in such close proximity as to obscure a clearly defined peak in the pattern. This influences the calculated mobility rate and in fact the various components in colostrum and in serum of mother and offspring are identical.

On the other hand Smith (1948) maintains that the immune lactoglobulin in bovine colostrum differs from the γ_2 globulin in serum in that the former has a mobility almost twice that of the latter. There appears to be no valid reason for

this conclusion as there is a considerable amount of a component having the mobility of serum γ_2 globulin present in colostrum, as shown very clearly in the electrophoresis pattern of bovine colostrum above. Two possible explanations can be offered for the difference of opinion.

Firstly, if the component here called α , (Smith, T component) is present in greater amounts than γ_2 , the former will obscure the latter in the diagram with the result that a spread out T or γ_1 peak is obtained with no definite summit which can be attributed to γ_2 .

Secondly, Perlmann and Kaufman (1949) have shown that the addition of acetic acid to serum has the property of denaturing the protein components irreversibly. This occurs at pH's below 4. Among different effects, it has the property of reducing the γ_2 globulin concentration with the formation of a component of migratory velocity intermediate between the α and β globulins. Smith removed the casein from the colostrum by iso-electric precipitation. This is done by the addition of dilute acid to the colostrum until a pH of 4.5 is reached at which point the casein is precipitated. Using this technique local variations of pH may occur in the solution during mixing with the subsequent reduction of the γ_2 globulin.

In veterinary practice where living attenuated viruses are used for immunization of domestic animals this transmitted passive immunity is of great importance. In fact it may be the dominant factor in elaborating a breeding programme. As the passive immunity wanes there occurs a period where the vaccine virus with its loss of invasiveness would be eliminated without multiplication and without durable active immunity production, yet at that stage the animal would succumb to natural virulent infection. On the other hand the protection of newborn lambs against fatal lamb dysentery intoxication is based upon immunization of the ewe before birth. The prime essential of each lamb having colostrum available for its first meal is stressed by the present study. This principle of maternal immunization may have wider application in the future in the control of the diseases of the newborn.

In human practice the same conception is valid since the immunity of the infant is mirrored in the maternal immunity even though the transmission of that immunity is entirely transplacental.

Summary and Conclusions.

Since there is no reason to doubt the generally accepted conception that the concentration of γ globulins is an accurate index of humoral immunity, it may be concluded that in bovines there is no transplacental transmission of specific immunity and that the acquired passive immunity of the calf results from the ingestion and absorption of the globulins present in high concentration in the colostrum. The human acquires its transmitted passive immunity exclusively *in utero* to a degree correlated with the immunity of its mother. This immunity is not influenced by the ingestion of colostrum from which the γ globulin component is absent.

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