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CLOTTING ACTIVITY OF MATERNAL AND FOETAL SHEEP BLOOD

BY P. FANTL AND H. A. WARD

From Baker Medical Research Institute, Alfred Hospital, Melbourne, Australia

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Most of the blood clotting factors are proteins and are produced by the liver. However, it is undecided where Factor VIII (antihaemophilic factor) is produced. (Nomenclature recommended by the International Committee for the Standardization of the Nomenclature of Blood Clotting Factors (*Brit. med. J.* 1959, i, 1292–1294) has been adopted in this paper.) Foetal liver lacks certain enzymes essential for protein synthesis, namely, those which hydroxylate phenylalanine and oxidize tyrosine (Kenney, Reem & Kretchmer, 1958). Further, γ-globulins and antibodies, which are produced by extra-hepatic tissues (Coons, Leduc & Connolly, 1955; Dancis, Braverman & Lind, 1957; Ortega & Mellors, 1957), are either absent, or present in only very low concentrations, in the foetal plasma of some species (Moore, Du Pan & Buxton, 1949). If, as evidence suggests, the foetal organism cannot form antibodies it may be possible to introduce into it cells which produce Factor VIII in the hope that they will survive and produce Factor VIII in the growing child. If grafting of Factor-VIII-producing cells were successful in new-born male infants of mothers who are α-haemophilia carriers it should then be possible to prevent them from becoming haemophiliacs.

It is therefore essential to know the activity of clotting factors of foetal blood. Such data from sheep are given here.

METHODS

Healthy ewes of known date of conception were used. They were of Merino breed and were mated with Merino rams. The gestation period is 144–160 days. Seventeen animals were available but not all tests were carried out on each animal. The pregnant ewes at varying stages of gestation were anaesthetized with intravenous Nembutal or Pentothal (pentobarbitone, thiopentone; Abbott Laboratories) and the foetuses exposed by Caesarian section. With siliconed syringes blood was withdrawn from one of the cord vessels and from the jugular vein of the ewe.

Plasma was obtained by mixing 9 volumes of blood with 1 volume of 0·1 M sodium oxalate and centrifuging at 2000 *g* at 6° C for 20 min. Plasma specimens were kept on ice until tested. The euglobin fraction of plasma was prepared as described by Fantl & Ward (1957).

Serum for the thromboplastin generation test was obtained by allowing blood to clot at 37° C for 1 hr. However, in order to obtain serum free of prothrombin it was necessary to clot 2·5 ml. blood in the presence of 0·1 g of glass-wool.

Brain was dehydrated with acetone according to the method of Quick (1940) and a suspension (3 or 6 %) of the dry powder in 0·145 M-NaCl was prepared by frequent grinding at 37° C during 2 hr in a glass tube with a pestle.

Clotting time was measured at 37° C on 1·5 ml. of blood in glass and in siliconed tubes.

One-stage prothrombin tests were performed, (*a*) with human and sheep brain extracts according to the method of Quick (1938); (*b*) with Russell's viper venom and phospholipid essentially according to the procedure of Hobson & Witts (1941). Prothrombin assays were carried out by the technique of Fantl (1954). Two procedures were used: (*a*) with human or sheep brain suspension; (*b*) the brain suspension was replaced by Russell's viper venom and phospholipid.

In preliminary experiments it was noted that the thrombin yield depended upon plasma concentration in the reaction mixture. The lowest plasma concentration gave the highest yield. It could be shown that catechol, which is used in the assay procedure to counteract antithrombin activity, did not affect the yield in the concentration employed. Further, the plasma euglobulin fraction, which is free from antithrombin, behaved similarly to plasma in the prothrombin assays.

Factor V was determined by the ability of the test plasma to shorten the clotting time of oxalated human plasma stored for 14 days at room temperature, the pH being adjusted to 7·4 with carbon dioxide before use. The test was similar to that of Quick & Stefanini (1948).

The combined activity of Factor VII and Prower-Stuart factor in serum was measured according to the procedure of de Vries, Alexander & Goldstein (1949).

Thromboplastin generation tests were performed at 28° C essentially as described by Biggs & Douglas (1953), except that oxalated plasma treated with $BaSO_4$ at 6° C for 30 min (referred to hereafter as Ba-plasma) was used and a phospholipid suspension in veronal buffer, pH 7·4, made according to the method of Fantl (1958) and containing 140 μg phospholipids, was substituted for platelets.

Partial thromboplastin times were determined by the method of Langdell, Wagner & Brinkhous (1953) modified as follows: a mixture of 0·2 ml. oxalated plasma, 0·1 ml. phospholipid suspension in veronal buffer, pH 7·4, and 0·05 ml. 0·145 M-NaCl or the material to be tested were incubated at 37° C for 1 min; 0·2 ml. 0·025 M-$CaCl_2$ was then blown into the mixture and the clotting time observed.

Assay of Factor VIII was carried out with oxalated plasma from a patient with a congenital complete deficiency of Factor VIII, to which dilutions of sheep or human Ba-plasma were added in either the thromboplastin generation test or the partial thromboplastin time test; similar clotting times were taken to indicate similar factor VIII activities. With both tests it is possible to determine Factor VIII activity which differs by more than 50 % from that of normal human plasma, the concentrations of other factors being equal.

Factor IX activity was measured with the thromboplastin generation test of Biggs & Douglas (1953), with sheep Ba-plasma and serum in the following way: clotting times obtained with 0·06 ml. pregnant ewe serum were taken to indicate 100 % Factor IX activity. A series of dilutions of ewe serum in 0·145 M-NaCl was prepared and that dilution which gave the same clotting time as 0·06 ml. foetal serum was taken as a measure of foetal concentration of Factor IX in terms of maternal concentration. Such a technique will only give accurate results if (*a*) the dilution of the test serum is not very high, and (*b*) it is assumed that other serum factors apart from Factor IX required for thromboplastin formation are present in adequate concentration. It was necessary to adopt the above technique because Factor-IX-deficient human plasma or serum in combination with foetal sheep serum gave a lower Factor IX concentration for the foetal sheep serum

than did homologous mixtures. The thromboplastin generation test is not very sensitive to changes in Factor IX activity, deviations of less than approximately 80% from normal not being detectable.

Human, sheep and bovine thrombin preparations were made as described by Fantl (1958).

Dialysis was carried out at 3° C in cellulose tubing suspended in a solution of 0·02 M trisodium citrate, 0·025 M sodium chloride and $1{\cdot}33 \times 10^{-4}$ M citric acid, pH 7·3, I approximately 0·145. The thrombin preparations were dialysed for 44 hr, at least one change of solution being made during this period, and the plasmata for 21 hr, with at least two changes of solution.

Nitrogen (N) was determined by a micro-Kjeldahl procedure.

Fibrinogen was determined as fibrin in 1 ml. oxalated plasma by addition of 1 ml. 0·025 M-$CaCl_2$ and 1 ml. of a thrombin preparation. The N content of the clot was determined after washing in 0·145 M-NaCl and was converted to fibrin by a factor of 5·92.

Plasma protein was estimated by determination of N in trichloroacetic-acid-insoluble material and conversion to protein by a factor of 6·25.

Moving-boundary electrophoresis was carried out in a Hilger Tiselius apparatus, in veronal-citrate buffer, pH 8·5, $I = 0{\cdot}05$, at 5° C, or in a Perkin–Elmer Model 38A Tiselius apparatus with an interferometric optical system, in veronal buffer, pH 8·6, $I = 0{\cdot}1$, at 4° C.

Platelets were counted by a technique similar to that of Feissly & Lüdin (1949).

RESULTS

The thromboplastin components in the blood of pregnant ewes and their foetuses

Results of the estimation of Factor VIII activity in the plasma of ewes and their foetuses are given in Table 1.

TABLE 1. Factor VIII activity in plasma of pregnant ewes and their foetuses expressed in terms of an arbitrary activity of 100 for human plasma

Gestation time (days)	86	99	128	128	134	142	145
Pregnant ewe plasma (a)	2500	1000	350	500	900	1200	650
Foetal plasma (b)	200	100	200	400	200	500	750
$b/a \times 100$	8	10	57	80	22	42	115

It is apparent from these results that a very low Factor VIII activity was present in foetal plasma at a gestation time of 86 days, and it increased during pregnancy. The activities given in the table are expressed in terms of normal human plasma which is arbitrarily taken to have an activity of 100. The experiments were carried out during several seasons and the human plasma used as a comparison was not taken from the same person in each case. As the Factor VIII activity of normal human plasma varies between 80 and 200 this variation is reflected in the potencies of the sheep plasmata. Plasma of normal non-pregnant sheep varied in Factor VIII activity from 450 to 1000. The values found for pregnant ewes showed similar variation. It will be noted that the lowest Factor VIII activity of foetal sheep plasma was of the same order as that of adult human plasma. Additional information on the Factor VIII activity of foetal plasma is supplied by thromboplastin generation tests in which mixtures of either pregnant ewe Ba-plasma or foetal Ba-plasma with ewe serum or

foetal serum were used. Results are given in Table 2, and show that, with the same serum, Ba-plasma of ewe or foetus gave similar clotting times. This indicates that the Factor VIII activity of foetal sheep plasma is not significantly lower than that of the maternal plasma.

The results given in Table 1, obtained from experiments carried out with heterologous plasma mixtures, showed lower Factor VIII activity for foetal sheep plasma than those in Table 2. Apparently Factor VIII of foetal sheep plasma has less affinity to the other components of the thromboplastin complex of human plasma than to those of sheep plasma.

TABLE 2. Thromboplastin generation test of Ba-plasma of pregnant ewe and foetus and homologous serum

Gestation time (days)	Serum	Ba-plasma	Minimum clotting time (sec)	Incubation time (min) for minimum clotting time
86	Pregnant ewe	Pregnant ewe	13	4
		Foetus	12	< 3
	Foetus	Pregnant ewe	15	< 2·5
		Foetus	16	< 3
142	Pregnant ewe	Pregnant ewe	12	< 3
		Foetus	12	< 3
	Foetus	Pregnant ewe	18	5
		Foetus	18	4·5

TABLE 3. Factor IX activity in foetal sheep serum expressed as percentage of maternal serum activity in thromboplastin generation test

Gestation time (days)	65	72	77	86	92	99	128	128	145
Foetal Factor IX activity	5	15	15	25	25	30	20	10	30

Results of the estimation of Factor IX activity in the serum of foetuses are given in Table 3.

This table shows that at all periods of gestation the foetal serum has a considerably lower activity than that of the mother.

The prothrombin complex in sheep blood

The prothrombin activity of plasma, which includes the activity of accessory factors for thrombin formation, was determined in one-stage tests in the presence of homologous and heterologous brain extracts and also with Russell's viper venom and phospholipid. Results are summarized in Table 4. This shows that a brain extract gave shortest clotting times with homologous plasma. Human plasma was less reactive with sheep brain than was sheep plasma with human brain. Foetal sheep plasma gave longer clotting times than maternal plasma with both sheep and human brain extracts. Russell's viper venom did not differentiate sig-

nificantly between the species although somewhat longer clotting times with foetal plasma were observed.

In Table 5 are shown one-stage prothrombin times and prothrombin assays related to the age of the foetus. From these results it is seen that the one-stage prothrombin time of foetal plasma was at all gestation times longer than that of the maternal plasma. Prothrombin assays indicated a very low prothrombin concentration in foetal plasma at an age of 70 days rising to 60 % of the maternal plasma concentration towards the

TABLE 4. One-stage prothrombin times (sec)

Oxalated plasma of	Human brain	Sheep brain	Russell's viper venom + phospholipid
Man	16 (15–17)	27 (22–32)	7·5 (7–8·5)
Pregnant ewe	18 (17–19)	10·5 (8·5–13)	8·5 (7–10)
Foetal sheep	23 (19–26)	13·5 (11·5–18)	10 (7·5–11·5)

Means (ranges in parentheses).

TABLE 5. One-stage prothrombin times and prothrombin concentrations in plasma of pregnant ewes and their foetuses

Gestation time (days) ...		70	77	92	98	98	98	128	128	134	145
One-stage prothrombin time (sec)*	Pregnant ewe	10	8·5	10·5	10	10	11	9	10·5	13	12
	Foetus	17·5	14·5	12·5	12	13	11·5	11·5	15·5	14	13·5
Prothrombin† concentration in plasma (units/ml.)	Pregnant ewe (*a*)	400	360	260	340	460	250	430	540	370	430
	Foetus (*b*)	65	50	130	120	120	60	200	240	160	260
$b/a \times 100$		16	14	50	35	26	24	47	44	43	60

* Sheep brain extract and 0·01 M-$CaCl_2$ were used.

† The assays were carried out with sheep brain extract, and in some cases with 5 μg Russell's viper venom and 25 μg phospholipid in place of the brain extract

end of gestation. It was established that the values obtained for prothrombin concentration in foetal plasma represented the true prothrombin level, since addition of the accessory factors—Factor V in ewe Ba-plasma and Factor VII and Prower–Stuart factor in ewe serum—did not increase the thrombin yield. For comparison the prothrombin level found for non-pregnant sheep was 280–460 units/ml. plasma and for man 480–640 units/ml. plasma.

For conversion of prothrombin into thrombin adequate amounts of Factor V are essential. Factor V activities of plasma of pregnant ewes and their foetuses are given in Table 6. It is apparent from Table 6 that at all gestation times studied the Factor V activity of foetal plasma was approximately the same as that of the ewe. Normal human plasma showed considerably lower activity.

In addition to Factor V two more plasma factors are required for efficient thrombin formation from prothrombin; these are Factor VII and

the Prower–Stuart factor. They were determined together by the ability to shorten the clotting time of diluted sheep plasma in the presence of sheep brain extract. Results are given in Table 7 from which it can be seen that Factor VII activity of foetal serum runs roughly parallel to prothrombin concentration.

TABLE 6. Factor V activity in plasma of pregnant ewes and their foetuses expressed in terms of an arbitrary activity of 100 for human plasma

Gestation time (days) ...	85	99	128	128	134	145
Pregnant ewe plasma ...	750	350	500	650	1000	500
Foetal plasma ...	750	250	350	750	700	1000

TABLE 7. Combined Factor VII and Prower–Stuart factor activity in serum of foetal sheep expressed as percentage of maternal serum activity. (Prothrombin concentrations in the respective plasmata, expressed as percentage of maternal plasma prothrombin concentration, are given for comparison)

Gestation time (days)	72	86	99	128	145
Factor VII + Prower-Stuart factor	20	50	50	30	50
Prothrombin	—	40	36	47	60

Fibrinogen concentration and activity of plasma of ewe and foetus

Fibrinogen and total protein concentrations of plasma are recorded in Table 8, from which it can be seen that at a foetal age of 70 days the fibrinogen concentration in foetal plasma was 16 % of that of the maternal plasma and near term had reached 83 % of the maternal level. Over the same interval the total protein concentration of the foetal plasma increased from 34 to 90 % of that of the mother. These results are in agreement with the findings of Alexander, Nixon, Widdas & Wohlzogen (1958). Plasma of non-pregnant sheep contained 200–350 mg fibrinogen/100 ml.

The reactivity of the fibrinogen of human, pregnant ewe and foetal sheep plasmata was compared with different thrombin preparations under a variety of experimental conditions. The results are summarized in Table 9. These results were obtained with fresh oxalated plasmata and also with dialysed plasmata. In the latter case maternal and foetal specimens were used from three different ewes of gestation times 128, 128 and 134 days.

It can be seen that with human and pregnant ewe plasmata the various thrombin preparations showed only small differences in activity. However a calcium-free human thrombin preparation gave considerably lower activity with foetal sheep plasma than with human or pregnant ewe plasma.

The addition of calcium ions had a greater shortening influence on the thrombin clotting time of the foetal plasma than on the other two plasmata. Thus in the presence of optimal calcium-ion concentration human throm-

bin produced practically identical clotting times in human, ewe and foetal plasmata. Although addition of calcium ions also shortened the thrombin clotting times when sheep or bovine thrombin was used the effect was not so pronounced as in the case of human thrombin.

The thrombin clotting time is dependent on fibrinogen concentration, but over the range of fibrinogen concentrations from 150 to 400 mg/100 ml. it does not vary significantly. Since all the plasmata used in the experiments shown in Table 9 had fibrinogen concentrations within this range it is unlikely that any differences in thrombin clotting time are due to markedly different fibrinogen levels.

TABLE 8. Fibrinogen and protein concentration in plasma of pregnant ewes and their foetuses

Gestation time (days) ...		70	77	86	98–99 (4)*	128 (2)*	134	142	145
Fibrinogen concentration in plasma (mg/100 ml.)	Ewe (*a*)	310	330	530	315–410	235–425	430	440	310
	Foetus (*b*)	50	70	155	140–210	195–255	145	225	230
$b/a \times 100$		16	21	29	34–67	60–83	34	51	74
Total protein concentration in plasma (g/100 ml.)	Ewe (*c*)	5·8	6·2	7·6	7·2–8·2	6·5–7·5	8·3	7·7	5·9
	Foetus (*d*)	2·0	2·4	2·7	2·8–3·2	4·5–4·6	3·8	4·8	5·3
$d/c \times 100$		34	39	36	35–42	60–71	46	62	90

* Where determinations were made on more than one ewe and foetus of a given gestation period the number of pregnant ewes is stated in parentheses.

TABLE 9. Thrombin activity with plasmata of man, pregnant ewe and foetal sheep

Plasma	Addition	Thrombin: Human	Thrombin: Bovine	Thrombin: Sheep
Human	NaCl	1	1	1
	$CaCl_2$	2·7	3·4	2·0
Pregnant ewe	NaCl	1	1·7	1·1
	$CaCl_2$	2·7	3·6	2·2
Foetal sheep	NaCl	0·2	1·5	1·1
	$CaCl_2$	2·7	4·0	2·3

0·2 ml. of either 0·075 M-NaCl or 0·025 M-$CaCl_2$ was added to 0·1 ml. plasma and 0·1 ml. veronal buffer, pH 7·3. 0·1 ml. thrombin preparation was then added at 36° C. Clotting times were converted into thrombin units. The results were recalculated taking the result obtained with human plasma with addition of NaCl as 1. Comparisons can only be made within each column.

The observations indicate that fibrinogen of foetal sheep plasma has properties different from that of maternal plasma. This is also seen in the behaviour of the blood clot. Whereas maternal sheep blood showed good clot retraction and little erythrocyte fall-out from the clot in a subsequent incubation period, foetal blood clots showed little or no retraction and a high fall-out of erythrocytes. The poor clot retraction could be due to a deficiency in the foetal platelets which, however, were present in

adequate number, as can be seen from Table 10. The erythrocyte fall-out is probably due to a structural weakness of the foetal fibrin which is unable to retain the erythrocyte mass. Fibrinolysis, which could be a cause of this high fall-out from the clot, was not detected in the foetal blood.

Whole blood clotting time

Determinations of whole blood clotting time are presented in Table 11 and show very little difference between foetal and maternal blood. The somewhat shorter clotting times of foetal blood are probably due to contamination of foetal blood with tisue thromboplastin during collection. The fact that clotting times in silicone were longer than those in glass for both maternal and foetal blood suggests that in both cases adequate concentrations of the contact factor (Hageman factor) are present.

TABLE 10. Platelet counts in blood of pregnant ewes and their foetuses

Gestation time (days) ...		65	72	77	86	92	98	98	128	128	134	145
Platelets $\times 10^{-3}$ per mm^3 blood	Ewe	420	430	480	640	610	480	450	550	790	430	630
	Foetus	200	240	470	370	560	950	670	780	700	580	820

TABLE 11. Clotting time (min) in glass and in silicone of blood from pregnant ewes and their foetuses

Gestation time (days) ...		70	92	98*	99	128	134	145
Pregnant ewe	Glass	7	7	7–13	21	12	15	—
	Silicone	—	—	—	38	73	35	—
Foetus	Glass	7	6	6–7	—	15	14	13
	Silicone	—	—	—	—	50	26	55

* Determinations were made on the blood from three pregnant ewes and foetuses of 98 days gestation.

Electrophoresis of plasma and serum

γ-Globulins are produced by the reticulo-endothelial system and it is possible that this is also the site for Factor VIII synthesis. Therefore electrophoresis of maternal and foetal sheep plasmata was carried out. A copy of a photographic schlieren pattern of both plasma and serum is shown in Fig. 1. The electrophoretic patterns indicate that the foetal plasma and serum were free from γ-globulin (component γ). Minor variations from the patterns in Fig. 1 were found on electrophoresis of other pregnant ewe and foetal sheep plasmata and sera, and from examination of some foetal serum patterns obtained by use of an interferometric optical system it appeared that a trace of γ-globulin may have been present.

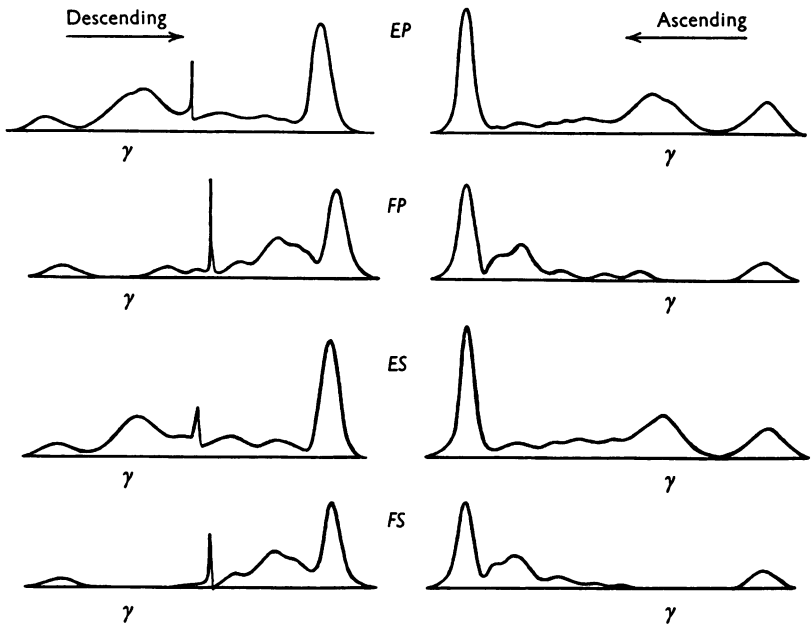

Fig. 1. Tracings of schlieren electrophoretic patterns of plasmata and sera from pregnant ewe and foetus of 98 days gestation. *EP* ewe plasma, *FP* foetal plasma, *ES* ewe serum, *FS* foetal serum. Conditions: veronal-citrate buffer, pH 8·5, $I = 0{\cdot}05$, 5° C. The patterns on the right are from the ascending boundaries, those on the left from the descending boundaries.

DISCUSSION

Regarding the presence of clotting factors in foetal blood, the possibility of production within the foetal organism and circulation or of transfer from the maternal circulation has to be considered. It cannot be stated whether Factor VIII is produced by the foetus or placenta, or is transferred from the ewe to the foetus. Some evidence for the production of Factor VIII in the foetal organism is given by the fact that Factor VIII in foetal plasma has a higher affinity, relative to that of Factor VIII in the maternal plasma, for the other components of the blood thromboplastin complex when these are homologous than when they are of human origin. It is interesting to note that the Factor VIII activity of the plasma of the youngest foetus (86 days) was as high as that of plasma of adult man.

The present experiments do not indicate the source of Factor VIII in plasma. A reduction of Factor VIII in experimental animals has been reported to occur following administration of ethionine (Pool & Spaet, 1954) and liver damage by hepatotoxins (Penick, Roberts, Webster & Brinkhous, 1958). This could be due to interference with protein synthesis

and does not indicate a specific site for Factor VIII synthesis, which may occur in the reticulo–endothelial system (Pool & Spaet, 1954). γ-Globulins were absent from foetal sheep serum, or at most, present in trace amounts. Therefore the reticulo-endothelial system of the sheep foetus is not fully developed, but while it is not able to produce γ-globulins it may be able to perform some of the other functions of the adult reticulo-endothelial system.

Evidence that synthesis of Factor VIII is not associated with γ-globulin synthesis in man is provided by the finding of Frick & Good (1956) that agammaglobulinaemia is not accompanied by reduced levels of Factor VIII.

The comparatively high Factor V activity of foetal plasma is remarkable. The available evidence indicates that this factor is produced by the liver in adults (Sykes, Seegers & Ware, 1948) but the source of Factor V in foetal plasma is undecided. In view of the fact that Factor V activity of foetal sheep plasma is of the same order as that of the pregnant ewe it is possible that Factor V is transferred from the maternal circulation to the foetus. Quick, Murat, Hussey & Burgess (1952) and Fresh, Ferguson, Stamey, Morgan & Lewis (1957) found Factor V levels in cord blood from new-born infants to be equal to or greater than the normal adult human level. In both full term and premature new-borns van Creveld, Paulssen & Teng (1952) observed Factor V concentrations in the normal adult range.

The prothrombin concentration of foetal sheep plasma was very low at 70 days and rose to 60% of that of the maternal plasma towards the end of gestation.

In full-term new-born humans mean plasma prothrombin concentrations varying from approximately 25 to 45% of the normal adult mean have been reported (Brinkhous, Smith & Warner, 1937; Loeliger & Koller, 1952; van Creveld, Paulssen & Teng, 1952; Fresh *et al.* 1957).

In foetal sheep serum the combined activity of Factor VII and Prower-Stuart factor, which were not determined separately in the present study, was found to be similar to the concentration of prothrombin and there was some parallelism between the levels of these factors and foetal age. Larrieu, Soulier & Minkowski (1952), van Creveld, Paulssen, Ens, van der Meij, Versteeg & Versteegh (1954), and Fresh *et al.* (1957) found the mean Factor VII content of cord blood from full-term infants to be approximately 40% of the mean normal adult level.

The fact that the activity of prothrombin, Factor VII and Prower-Stuart factor in foetal sheep blood is lower than in the maternal blood and the approximate correlation between the foetal blood levels and age may reflect the diminished ability of the foetal liver to produce these clotting factors.

Prothrombin and Factors VII and IX have a number of properties in common and may be chemically related. The much lower activity of Factor IX in foetal serum is therefore surprising. Perhaps the enzyme system required for synthesis of Factor IX is more complex than those involved in prothrombin and Factor VII synthesis and is not well developed in the foetal liver. Barkhan (1957) reported that sera from cord blood of new-born infants had a Factor IX activity of 19–91% (mean 49%) compared with a mean activity of 108% for normal human adult serum.

The fibrinogen concentration in foetal sheep plasma was 50 mg/100 ml. in the youngest foetus on which a determination was carried out (70 days); near term it had reached 230 mg/100 ml. which is approximately 80% of the concentration in ewe plasma.

In new-born lambs Field, Spero & Link (1951) found an average of 226 mg fibrinogen and 6·15 g total protein/100 ml. plasma.

In eight human foetuses, from 15 to 20 weeks of age, Vahlquist, Westberg & de las Heras (1953) found 50–290 (mean 180) mg fibrinogen/100 ml. plasma, and 140–700 (mean 390) mg/100 ml. plasma in the cord blood of fourteen full-term infants. Taylor (1957) has summarized a number of studies on fibrinogen concentration in the normal new-born infant, and has reported his own findings of 192–398 (mean 231) mg/100 ml. plasma compared with 305–674 (mean 440) mg/100 ml. in maternal plasma and 217–354 (mean 280) mg/100 ml. for normal adults.

Foetal sheep fibrinogen showed the same behaviour as maternal fibrinogen towards sheep and bovine thrombin but gave longer clotting times with thrombin of human origin. However, on addition of calcium ions, which shortened the clotting time in all cases, this different behaviour of human thrombin with foetal sheep fibrinogen was abolished.

A prolonged thrombin clotting time has been noticed with fibrinogen of new-born infants (Biggs, 1951; Larrieu *et al.* 1952; Ferguson, Fresh & Lewis, 1955; Vecchio & Schettini, 1957), and Jayle, Badin & Ottolenghi-Preti (1949) and Burstein, Lewi & Walter (1954) have found that certain physical properties of the fibrin clot from the plasma of new-born infants are different from those of normal adult fibrin. The finding of a difference in activity of the foetal and maternal fibrinogen indicates that fibrinogen in the foetus is produced in the foetal circulation rather than being transferred from the ewe.

Although the placenta of the sheep is of the syndesmochorial type and thus differs structurally from the human placenta, which is of the more permeable haemochorial type, it appears from a comparison of the presented results with the findings in human blood that the respective activities of the clotting factors in the blood of mother, foetus and normal

adult are similar in the two species. Certain speculations about blood coagulation in the human infant would therefore seem justified.

Determination of Factors VII, IX, and prothrombin in the blood of a new-born infant does not permit one to predict whether a haemorrhagic tendency due to a deficiency of one or more of these factors will develop later in life as the activity of these factors is low in the normal new-born and increases with age. On the other hand the activity of Factor VIII in the blood of the normal new-born is as high as in the normal adult (Fresh, Ferguson & Lewis, 1956; van Creveld, Nagel, Nijenhuis, Miranda & Tjon Sien Kie, 1954).

We found 50–75 % of the normal adult factor VIII activity in the cord blood of a new-born baby in whose pedigree there was no record of Factor VIII deficiency. As far as we are aware no observations of Factor VIII levels in new-born infants of carriers of Factor VIII deficiency has yet been made, but it would seem that a low Factor VIII activity in the blood of a new-born infant would indicate a permanent Factor VIII deficiency and measures to induce Factor VIII production as suggested in the introduction would be justified.

SUMMARY

1. Factor VIII activity is detectable in foetal sheep plasma at an early stage of gestation and rises to adult levels towards the end of gestation. At all gestation times Factor VIII activity of foetal sheep plasma is at least as high as that of adult human plasma.

2. Factor V activity of foetal sheep plasma is at all gestation times as high as that of maternal plasma. Factor V activity of sheep plasma is higher than that of man.

3. Prothrombin activity of foetal sheep plasma is low at early stages of gestation and rises to approximately 60 % of that of maternal plasma near term. Normal sheep plasma contains approximately 70 % of the prothrombin of normal human plasma.

4. In the serum of foetal sheep, Factor VII and Prower–Stuart factor, measured together, show some parallelism with the prothrombin concentration of plasma.

5. Factor IX activity of foetal sheep serum is at all foetal ages low compared with that of the maternal serum.

6. Fibrinogen concentration of foetal sheep plasma is low at early stages of gestation and rises to approximately 80 % of that of the maternal plasma near term.

7. Foetal sheep fibrinogen shows different activity from that of the maternal fibrinogen.

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REFERENCES

ALEXANDER, D. P., NIXON, D. A., WIDDAS, W. F. & WOHLZOGEN, F. X. (1958). Renal function in the sheep foetus. *J. Physiol.* **140**, 14–22.

BARKHAN, P. (1957). Christmas-factor activity of cord blood. *Brit. J. Haemat.* **3**, 215–219.

BIGGS, R. (1951). *Prothrombin deficiency*, p. 55. Oxford: Blackwell.

BIGGS, R. & DOUGLAS, A. S. (1953). The thromboplastin generation test. *J. clin. Path.* **6**, 23–29.

BRINKHOUS, K. M., SMITH, H. P. & WARNER, E. D. (1937). Plasma prothrombin level in normal infancy and in hemorrhagic disease of the newborn. *Amer. J. med. Sci.* **193**, 475–480.

BURSTEIN, M., LEWI, S. & WALTER, P. (1954). Sur l'existence du fibrinogène foetal. *Le Sang*, **25**, 102–107.

COONS, A. H., LEDUC, E. H. & CONNOLLY, J. M. (1955). Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. exp. Med.* **102**, 49–60.

DANCIS, J., BRAVERMAN, N. & LIND, J. (1957). Plasma protein synthesis in the human fetus and placenta. *J. clin. Invest.* **36**, 398–404.

DE VRIES, A., ALEXANDER, B. & GOLDSTEIN, R. (1949). A factor in serum which accelerates the conversion of prothrombin to thrombin. I. Its determination and some physiologic and biochemical properties. *Blood*, **4**, 247–258.

FANTL, P. (1954). The use of substances depressing antithrombin activity in the assay of prothrombin. *Biochem. J.* **57**, 416–421.

FANTL, P. (1958). Thrombin and the thromboplastic activity of plasma. *Aust. J. exp. Biol. med. Sci.* **36**, 581–587.

FANTL, P. & WARD, H. A. (1957). Comparison of blood clotting in marsupials and man. *Aust. J. exp. Biol. med. Sci.* **35**, 209–224.

FEISSLY, R. & LÜDIN, H. (1949). Microscopie par contrastes de phases (III. Applications a l'hématologie). *Rev. Hémat.* **4**, 481–501.

FERGUSON, J. H., FRESH, J. W. & LEWIS, J. H. (1955). Blood-clotting studies on the newborn. *Fed. Proc.* **14**, 47–48.

FIELD, J. B., SPERO, L. & LINK, K. P. (1951). Prothrombin and fibrinogen deficiency in newborn pups and lambs. *Amer. J. Physiol.* **165**, 188–194.

FRESH, J. W., FERGUSON, J. H. & LEWIS, J. H. (1956). Blood-clotting studies in parturient women and the newborn. *Obstet. & Gynec.* **7**, 117–127.

FRESH, J. W., FERGUSON, J. H., STAMEY, C., MORGAN, F. M. & LEWIS, J. H. (1957). Blood prothrombin, proconvertin and proaccelerin in normal infancy: questionable relationships to vitamin K. *Pediatrics*, **19**, 241–251.

FRICK, P. G. & GOOD, R. A. (1956). Studies on agammaglobulinemia. VI. Hemostasis in patients with agammaglobulinemia. *Proc. Soc. exp. Biol., N.Y.*, **91**, 169–172.

HOBSON, F. & WITTS, L. J. (1941). Venom-lecithin reagent for the accelerated clotting test (prothrombin time). *J. Path. Bact.* **52**, 367–381.

JAYLE, M. F., BADIN, J. & OTTOLENGHI-PRETI, G. (1949). La cinétique de la coagulation sanguine. Étude critique de ses données, nouveau mode d'expression. Application pratique à l'étude des suites de couches et des phlébites du post-partum. *Ann. Biol. clin.* **7**, 311–330.

KENNEY, F. T., REEM, G. H. & KRETCHMER, N. (1958). Development of phenylalanine hydroxylase in mammalian liver. *Science*, **127**, 86.

LANGDELL, R. D., WAGNER, R. H. & BRINKHOUS, K. M. (1953). Effect of antihemophilic factor on one-stage clotting tests. A presumptive test for hemophilia and a simple one-stage antihemophilic factor assay procedure. *J. Lab. clin. Med.* **41**, 637–647.

LARRIEU, M. J., SOULIER, J. P. & MINKOWSKI, A. (1952). Le sang du cordon ombilical: étude complète de sa coagulabilité, comparison avec le sang maternel. *Études néo-natales*, **1**, 39–60.

LOELIGER, A. & KOLLER, F. (1952). Behaviour of factor VII and prothrombin in late pregnancy and in the newborn. *Acta haemat.* **7**, 157–161.

MOORE, D. H., DU PAN, R. M. & BUXTON, C. L. (1949). An electrophoretic study of maternal, fetal and infant sera. *Amer. J. Obstet. Gynec.* **57**, 312–322.

ORTEGA, L. G. & MELLORS, R. C. (1957). Cellular sites of formation of gamma globulin. *J. exp. Med.* **106**, 627–640.

PENICK, G. D., ROBERTS, H. R., WEBSTER, W. P. & BRINKHOUS, K. M. (1958). *Hemorrhagic* states secondary to intravascular clotting. *A.M.A. Arch. Path.* **66**, 708–714.

POOL, J. G. & SPAET, T. H. (1954). Ethionine-induced depression of plasma antihemophilic globulin in the rat. *Prpc. Soc. exp. Biol., N.Y.*, **87**, 54–57.

QUICK, A. J. (1938). The nature of the bleeding in jaundice. *J. Amer. med. Ass.* **110**, 1658–1662.

QUICK, A. J. (1940). The thromboplastin reagent for the determination of prothrombin. *Science*, **92**, 113–114.

QUICK, A. J., MURAT, L. G., HUSSEY, C. V. & BURGESS, G. F. (1952). The prothrombin in the newborn. *Surg. Gynec. Obstet.* **95**, 671–676.

QUICK, A. J. & STEFANINI, M. (1948). The concentration of the labile factor of the prothrombin complex in human, dog, and rabbit blood; its significance in the determination of prothrombin activity. *J. Lab. clin. Med.* **33**, 819–826.

SYKES, E. M., JR., SEEGERS, W. H. & WARE, A. G. (1948). Effect of acute liver damage on Ac-globulin activity of plasma. *Proc. Soc. exp. Biol., N.Y.*, **67**, 506–507.

TAYLOR, P. M. (1957). Concentrations of fibrinogen in the plasma of normal newborn infants. *Pediatrics*, **19**, 233–240.

VAHLQUIST, B., WESTBERG, V. & DE LAS HERAS, M. (1953). Prothrombin and fibrinogen values in the young human fetus. *Acta Soc. Med. upsalien.* **58**, 281–284.

VAN CREVELD, S., NAGEL, C. G. M., NIJENHUIS, J. H., MIRANDA, S. I. & TJON SIEN KIE (1954). Thromboplastin formation in the blood of the newborn. *Études Néo-natales*, **3**, 135–143.

VAN CREVELD, S., PAULSSEN, M. M. P., ENS, J. C., VAN DER MEIJ, C. A. M., VERSTEEG, P. & VERSTEEGH, E. T. B. (1954). Proconvertin content in the blood of newborn full-term and premature infants. *Études Néo-natales*, **3**, 53–61.

VAN CREVELD, S., PAULSSEN, M. M. P. & TENG, S. K. (1952). Prothrombin and accelerator globulin in the plasma of newborns under normal and pathological conditions. *Études Néo-natales*, **1**, 87–101.

VECCHIO, F. & SCHETTINI, F. (1957). Studio sull'emocoagulazione nel neonato. V. Tempo di trombina e fibrinogenemia. *Biochimica Applicata*, **4**, 173–180.