

## FIBRINOLYSIS IN NORMAL PREGNANCY

BY

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It is well known that a low level of circulating fibrinogen may be associated with bleeding in a variety of obstetric complications, as, for example, accidental haemorrhage. The actual cause, however, of the depletion of fibrinogen in these conditions is debatable (Moore, 1955), but one feature of such states is the frequent presence of a circulating proteolytic agent which is capable of destroying fibrin. It is possible that this agent is responsible for the bleeding, though attempts to demonstrate it have not always been successful.

One explanation of the failure to detect lysis is suggested by the work of Fearnley, Revill, and Tweed (1952), who showed that plasma fibrinolytic activity is thermolabile. This activity is quickly destroyed at room temperature, but can be preserved by keeping the plasma at ice temperature.

Such a precaution has been adopted in the present work, which is concerned with the behaviour of plasma fibrinolytic activity in normal pregnancy, labour, and the puerperium.

### Methods and Material

**Qualitative Test (Modified from Fearnley and Tweed, 1953).**—Bottles and syringes are carried to and from the wards in a container filled with ice. Further manipulations in the laboratory are carried out using ice-cold glassware and ice-cold reagents.

Venous blood is taken into a bottle containing sequestric acid as an anticoagulant and carried immediately to the laboratory. The blood is centrifuged at 3,000 r.p.m. for two minutes. Doubling dilutions of the plasma up to 1/16 are made with a veronal buffer solution (pH 7.35) containing merthiolate in a final concentration of 1/100,000. The neat plasma and dilutions are clotted immediately with sufficient thrombin to produce a clot in 30 seconds and placed in a water-bath at 37° C.

The test is read after 24 hours' incubation. If a clot is absent in any one tube the test is regarded as positive.

**Semi-quantitative Test (Modified from MacFarlane and Pilling, 1946).**—Doubling dilutions of the test plasma are made from 1/8 to 1/2,048. The diluent consists of veronal merthiolate buffer containing fibrinogen in an approximate concentration of 50 mg.%. The first tube (1/8) is a dilution of plasma with veronal merthiolate

buffer only; the concentration of the fibrinogen in this tube would lie between 40 mg. and 60 mg.%. In this way the concentration of fibrinogen is the same in all tubes, i.e., approximately 50 mg.%.

The tubes are clotted with thrombin as before and placed in a water-bath at 37° C. The whole procedure of the qualitative and semi-quantitative tests up to incubation is carried out within 15 minutes from the obtaining of the blood.

The tubes are examined after 24 hours' incubation and the titre expressed is the dilution of the last tube in which a clot is absent. Some tubes at a higher dilution show partial dissolution of the clot, but, since it is difficult to assess the degree of lysis, these tubes are not included in the titre expressed.

Repeated bacteriological tests demonstrated that the tubes of both tests remained sterile.

**Reagents.**—The following are required:

**Veronal Buffer Solution (pH 7.35).**—Sodium diethyl barbiturate 12.75 g., 5.67 g. sodium chloride, 430 ml. 0.1 N HCl, and 570 ml. distilled water.

**Thrombin.**—Thrombin (Roche) is dissolved in distilled water at a concentration of approximately 10 units/ml. and stored frozen. It is thawed immediately before use.

**Fibrinogen.**—The method of preparation is modified from that of Ware, Guest, and Seegers (1947). Fresh human plasma (sequestrene anticoagulant) is left standing at room temperature for several hours. It is then frozen overnight at -10° C. The following morning it is allowed to thaw slowly until a piece of ice the size of a grape remains. The plasma is spun quickly at 3,000 r.p.m. At the end of spinning a small piece of ice should still be present. The supernatant is discarded. The sediment includes fibrinogen and is washed once with ice-cold saline by centrifuging for one minute at 2,000 r.p.m. at room temperature. The supernatant is discarded and the precipitate dissolved in normal saline at 37° C. The solution is centrifuged for two minutes at 2,000 r.p.m., decanted, and freeze dried. Simple experiments demonstrated that this preparation of fibrinogen possesses neither fibrinolytic nor anti-fibrinolytic activity in the concentration used.

The patients from whom test samples were obtained were the ordinary out-patients and in-patients of the hospital. About 500 patients were examined by the qualitative technique and 400 of these were also tested by the semi-quantitative method. At the time of veni-

puncture all patients were normal. Of those examined during the antenatal period, over 90% remained normal and those examined during labour and the puerperium had had a normal pregnancy. Sequential tests on individual patients were not carried out.

Fifty-four normal non-pregnant women were tested as a control series.

**Results**

**Qualitative Test.**—The percentage incidence of lytic activity in non-pregnant females, at all stages of pregnancy, during labour, and the puerperium is given in Table I and Fig. 1.

The figure for non-pregnant females, i.e., 92%, corresponds closely to that of Fearnley and Tweed (1953), who, using a similar technique, found lysis in 50 out of 60 patients.

TABLE I  
INCIDENCE OF FIBRINOLYSIS IN NORMAL PREGNANCY

Group		Total No. of Patients	No. of Patients Showing Lysis
Non-pregnant .. .. .		54	50 (92.6%)
Gestation Period in Weeks	0 to 10 .. .. .	23	23 (100%)
	11 to 15 .. .. .	43	40 (93%)
	16 to 20 .. .. .	52	38 (73%)
	21 to 25 .. .. .	23	11 (47.8%)
	26 to 30 .. .. .	26	5 (19.2%)
	31 to 35 .. .. .	40	3 (7.5%)
	36 to labour .. .. .	53	6 (11.3%)
Labour	First and second stages ..	54	4 (7.4%)
	Third stage .. .. .	7	2 (29%)
Puerperium	0 to 3 hours .. .. .	31	24 (77.4%)
	3 hours to 8 days .. .. .	93	83 (89%)
	6 weeks .. .. .	13	11 (85%)

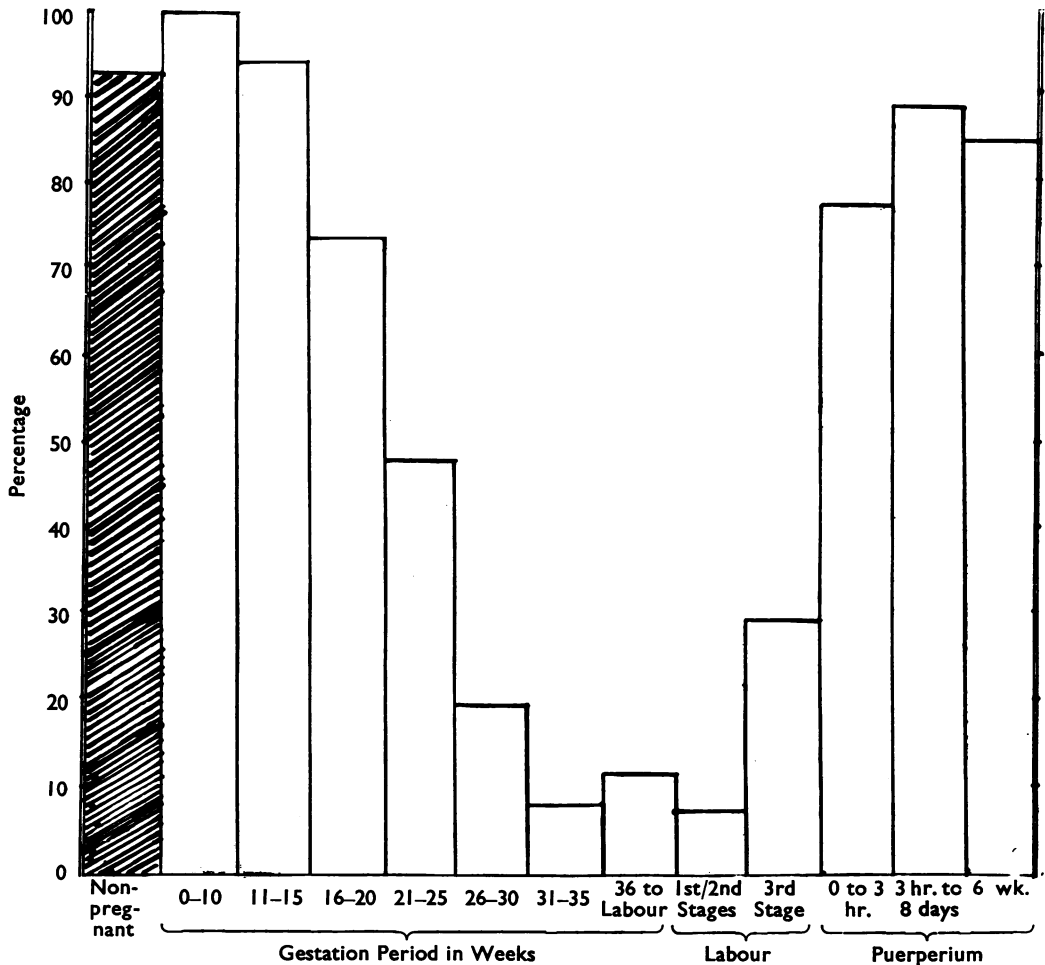


FIG. 1.—Incidence of fibrinolysis in normal pregnancy.

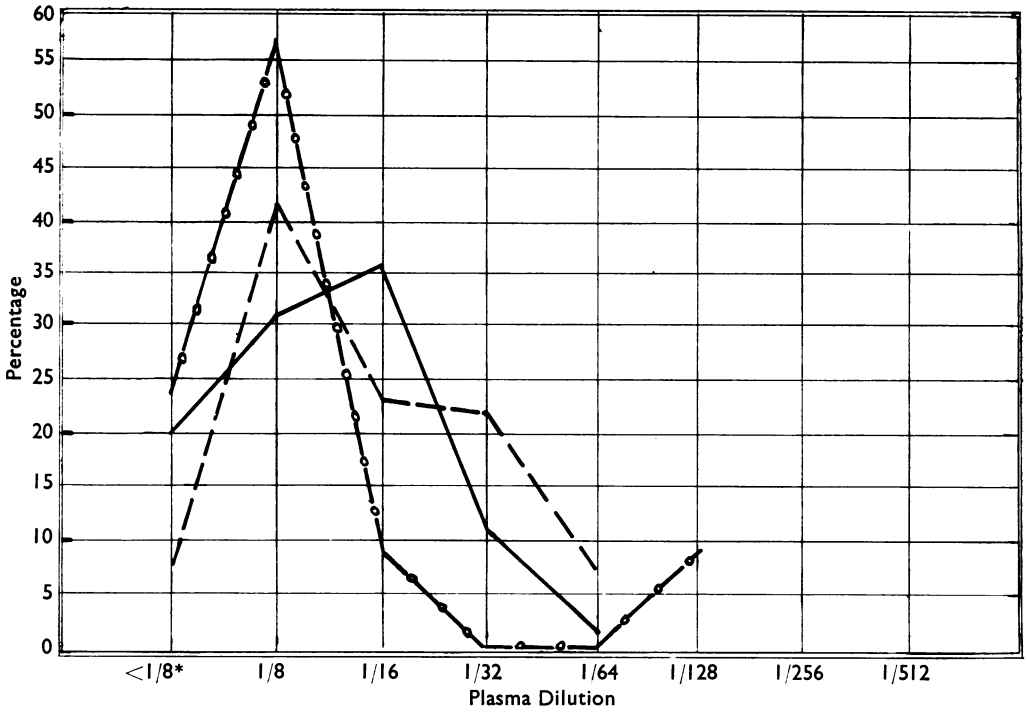


FIG. 2.—Distribution of semi-quantitative titres in antenatal period. — non-pregnant. - - - - antenatal: 0 to 20 weeks. —○—○—○ antenatal: 20 weeks to labour. \*  $\equiv <1/8$   $\equiv$  no dissolution of clot in the lowest dilution, but qualitative test is positive.

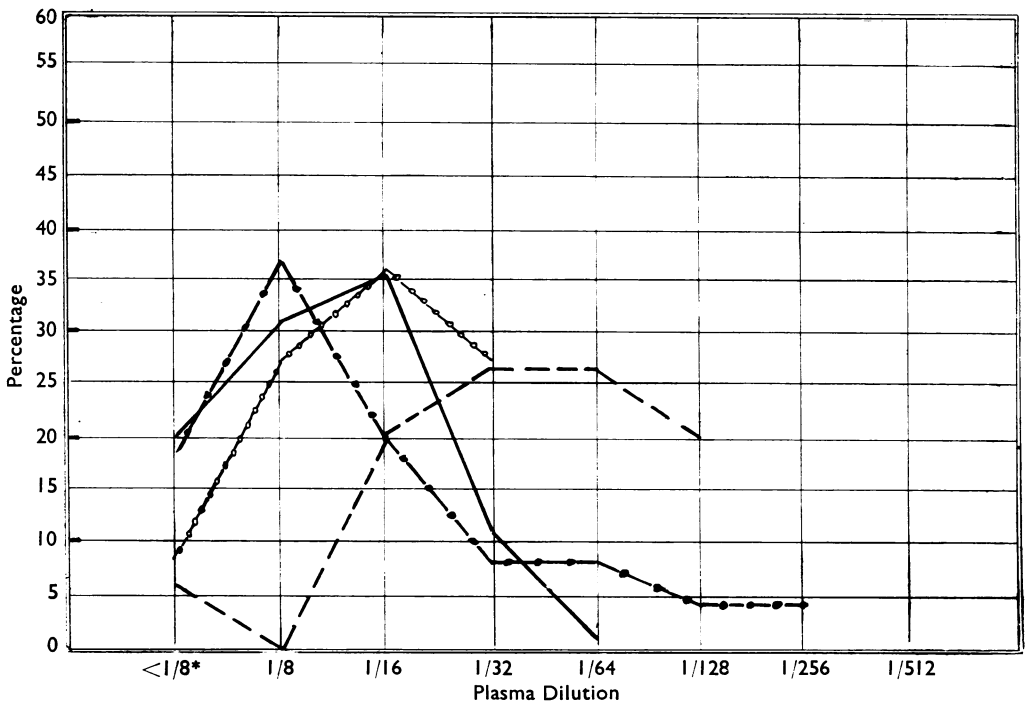


FIG. 3.—Distribution of semi-quantitative titres in puerperium. — non-pregnant. - - - - puerperium: 0 to 3 hours. - . - . - . puerperium: 3 hours to 8 days. —○—○—○ puerperium; at 6 weeks. \*  $\equiv <1/8$   $\equiv$  no dissolution of clot in the lowest dilution, but qualitative test is positive.

TABLE II  
DISTRIBUTION OF SEMI-QUANTITATIVE TITRES IN ANTENATAL PERIOD AND PUERPERIUM

Group	Total No. of Patients	No. of Patients Showing Lysis	Distribution of Titre							
			< 1/8*	1/8	1/16	1/32	1/64	1/128	1/256	1/512
Non-pregnant ..	48	45	9 (20%)	14 (31.1%)	16 (35.6%)	5 (11.1%)	1 (2.2%)	—	—	—
Gestation period in weeks:										
0 to 20 ..	118	101	7 (6.9%)	42 (41.6%)	23 (22.8%)	22 (21.8%)	7 (6.9%)	—	—	—
20 to labour ..	117	21	5 (23.8%)	12 (57.2%)	2 (9.5%)	—	—	2 (9.5%)	—	—
Puerperium:										
0 to 3 hours ..	20	15	1 (6.6%)	—	3 (20%)	4 (26.7%)	4 (26.7%)	3 (20%)	—	—
3 hours to 8 days ..	55	49	9 (18.4%)	18 (37%)	10 (20%)	4 (8.2%)	4 (8.2%)	2 (4.1%)	2 (4.1%)	—
6 weeks ..	13	11	1 (9.0%)	3 (27.3%)	4 (36.4%)	3 (27.3%)	—	—	—	—

\* ≡ < 1/8 ≡ no dissolution of clot in the lowest dilution, but qualitative test is positive.

The most interesting finding is the progressively falling incidence of lysis found after the 15th week of pregnancy, reaching the lowest value of about 10% during the last 10 weeks and the first two stages of labour. Equally startling is the rapid increase of incidence after delivery so that the percentage value three hours postpartum is about 75% and at 24 hours reaches a normal non-pregnant value of 90%. This value remains unchanged up to seven days and at six weeks postpartum.

**Semi-quantitative Test.**—It is realized that this test cannot be anything more than semi-quantitative, for although fibrinogen and thrombin remain relatively constant, plasma factors other than the fibrinolysin are also diluted. Nevertheless the results within the individual groups are so consistent as to suggest that the different titres do, in fact, represent different levels of lytic activity.

The actual test also behaved consistently. For example, in only six instances (out of about 400 estimations) did it occur that, when lysis was present in a high dilution, lytic activity was absent in tubes of a lower dilution.

The range of titre of lytic activity for non-pregnant women during pregnancy, labour, and the puerperium is given in Table II and Figs. 2 and 3. The important conclusions to be drawn are:

(a) The highest titre in the non-pregnant group is 1/64 and 98% of cases do not exceed 1/32. The values for the antenatal group of up to 20 weeks' gestation are about the same, i.e., a highest titre of 1/64, with 93% of cases not exceeding 1/32. In the group 20 weeks to labour, although the highest titre is 1/128, about 92% of cases have a value not exceeding 1/16. This would suggest that not only is the percentage incidence reduced during the last 20 weeks of pregnancy (Fig. 1) but that, when lysis is demonstrable, it tends to have a lower titre. Such a conclusion can only be tentative because of the fewer positive cases in the later weeks of pregnancy.

(b) It is apparent from Fig. 3 that the distribution of the titres at six weeks postpartum is the same as in the normal non-pregnant group. However, during the period three hours postpartum to eight days postpartum the highest titre reaches 1/256 with 84% of values less than 1/32. There is a suggestion, therefore, of a slight increase in activity although most cases fall within the normal range.

Nevertheless it is the distribution curve for the period 0 to three hours postpartum which shows the most obvious increase of titre. In this group, composed of only 15 cases, the range reaches 1/128, but seven cases, i.e., 47%, have a titre of 1/64 or more.

Of the 40 patients who were tested during labour, only two showed lysis and these gave titres of less than 1/8 and 1/16.

**Discussion**

Recent reports concerned with fibrinolysis in pregnancy give widely divergent results. Cervini and Ficola (1951), Margulis, Luzadre, and Hodgkinson (1954), and Niesert (1955) agree that there is a greater incidence of fibrinolysis in the puerperium than in the antenatal period. Ciculla and Luraschi (1953a and b) came to the opposite conclusion. These latter authors also investigated patients at different periods of gestation but unfortunately did not give details. Shea (1955) investigated fibrinolytic activity in 34 puerperal women and found activity in only two, and somewhat earlier MacFarlane and Biggs (1946) mention that they found no evidence of proteolytic activity in 137 normal women during pregnancy. None of these reports can be said to agree wholly with the present findings. The reason for the discrepancy is probably technical, for none of the above authors preserved lytic activity by keeping the plasma and reagents at ice temperature.

The results reported here are surprising, particularly those obtained during labour. It has been demonstrated that both exercise and stress increase lysis in normal men and women (Biggs, MacFarlane, and Pilling, 1947; Truelove, 1953). Labour may be assumed to involve both exercise and stress and yet lytic activity at the time is exceptional. Whether this inhibition, in the antenatal period as well as during labour, is due to a low concentration of fibrinolysin or to an increased antifibrinolysin is unknown and is being investigated at the present time.

The effect of fibrinogen concentration on lytic activity has been commented on by various workers. Apparently fibrinolysis *in vitro* decreases with an increase in the amount of fibrinogen (Shulman, 1952a; Bozzo, Piomelli, and Schettini, 1956). It is possible, therefore, that the high levels of plasma fibrinogen which may be found in pregnancy could explain the inhibition of lysis. However, in the puerperium, normal or high fibrinogen levels are associated with normal or enhanced lytic activity, and Shulman, too (1952b), found that fibrinogen levels *in vivo* had no influence on fibrinolytic activity.

Clearly many factors could have an influence, but two may be mentioned. Lipaemia, which is known to be increased in pregnancy, has been shown to exert an anti-fibrinolytic effect in men and non-pregnant women (Greig, 1956); and hormones, too, exert an influence on lysis in the experimental animal (Ungar, Damgaard, and Hummel, 1951). There is no definite evidence to link either of these two factors with fibrinolytic activity in pregnancy and their role can be no more than speculative.

Finally there is one practical point of importance. Whenever lytic activity is discovered in the puerperium it is often assumed that such activity is abnormal. It is clear from the present work that

lytic activity in the puerperium is a return to the normal physiological state of the non-pregnant woman and that therefore some kind of quantitative assessment is necessary in order to determine abnormal degrees of lysis.

### Summary

Qualitative and semi-quantitative assessments of fibrinolytic activity in normal pregnancy, labour, and puerperium and in a number of non-pregnant women have been carried out.

The results show a marked decrease in the activity in the later months of pregnancy and labour and a quick return to about the non-pregnant level immediately after delivery.

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