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Platelet Studies in Menstruation and Hemophilia: Total and Differential Counts, Disintegration Rates and Lipid Distributions.

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Few observations of any kind have been made on the platelets of menstruating women although Genell[1] called attention to the variations in platelet counts during menstruation. According to Birch[2, 3] partial control of the hemorrhages of hemophilia may be obtained by treatment with ovarian extract. She has pointed out that it is possible that the female carrier is protected from bleeding by her sex hormones. This possibility suggested that a comparative study of the platelets in hemophilia and in menstruation might reveal interesting facts. To this end total and differential counts, disintegration rates, and detailed lipid studies were made upon platelet preparations from the blood of normal women, between and during menstruations, and hemophilic children.

The technic of Olef[4] for platelet counting and determination of disintegration rate[5] * was chosen for these studies. In this indirect method, to prevent clumping and disintegration, a drop of sodium metaphosphate is placed on the site of the finger puncture so that the blood is mixed directly with the diluting fluid and does not contact the skin. After transferring to a paraffine cup, fresh preparations are made from the mixture for counting. A simultaneous erythrocyte count is also necessary to evaluate the absolute number of platelets. The platelets are differentiated into 3 groups according to size, using the red cell as a standard: Group I is one-fourth the size of the red cell, Group II is one-third, and Group III is one-half.

Observations were made on 10 normal women when not menstruating and 9 hemophilic children. Five of the women were studied

1 Genell, S., *J. Obst. and Gynec.*, Brit. Empire, 1936, **43**, 1124.

2 Birch, C. L., PROC. SOC. EXP. BIOL. AND MED., 1930-31, **28**, 752.

3 Birch, C. L., *J. A. M. A.*, 1932, **99**, 1566.

4 Olef, I., *J. Lab. and Clin. Med.*, 1935, **20**, 416.

5 Olef, I., *J. Lab. and Clin. Med.*, 1936, **22**, 128.

* Olef's technic for disintegration rate was modified slightly by placing the paraffine cup (containing the blood) in the incubator at 37°C for 6 hours, instead of in the refrigerator for 8 hours.

TABLE I.
Total and Differential Platelet Counts and Disintegration Rates.

	Untreated hemophiliacs		Normal Women			
			Non-Menstruating		Menstruating	
	Avg of 7 determinations on 4 subjects*	One subject	Avg of 5 determinations on 4 subjects	One subject	Avg of 7 determinations on 5 subjects	One subject
Clotting time		2 hr		11 min		20 min
Total platelet count in thousands:						
Initial		450		828		330
Incubated 6 hr		520		494		374
Disintegration rate	+5%	+16%	—41%	—40%	+2%	+13%
Differential platelet count:						
Group I:						
Initial		329		248		122
Incubated 6 hr		140		213		104
Increase or decrease	—25%	—57%	—30%	—14%	+9%	—15%
Group II:						
Initial		117		580		208
Incubated 6 hr		380		281		225
Increase or decrease	+62%	+225%	—34%	—52%	—3%	+8%
Group III:						
Initial		4.5		—		0
Incubated 6 hr		—		—		45

*Five of the hemophiliacs are not included in this tabulation because they had received treatment. They will be discussed in a later paper.

during menstruation. Sixty-five total and differential platelet counts and 20 disintegration rates were determined. Complete data are given in Table I upon one hemophilic child, one non-menstruating woman, and one menstruating woman, and the average percentages for the groups.

It may be seen that the non-menstruating women have a decided decrease in both Groups I and II while the hemophiliacs show a decrease in Group I and a marked increase in Group II. The disintegration rate figures obtained during menstruation fall between the normal and the hemophiliacs. The most striking similarity of the platelets of hemophilia and menstruation is found in the slower (+) disintegration rate.

The increase in Group II found in hemophilia may be due to the initial swelling that the platelets have undergone in the first stage of the process of normal disintegration. Ferguson,[6] in studying disintegration of normal platelets by means of the dark field, has observed that the first change is a swelling into a "spherule". Suspension of the process at this stage would explain an increase in the larger forms and a decrease in the smaller.

Since cephalin has been shown to play an important part in blood clotting, detailed lipid studies were made on the platelets of menstruating and non-menstruating women. Platelet samples were collected from the blood of 5 normal women during the first days of menstruation and were pooled for chemical analyses.† The lipid composition, together with comparative data on a pooled platelet sample collected from the blood of 5 normal women during the intermenstrual period,[7] is presented in Table II.

TABLE II.
Lipid Composition of Human Platelets Between and During Menstruations.

	Inter-Menstruation Avg for 5 subjects		Menstruation Avg for 5 subjects	
	Dry weight of platelets %	Total lipid %	Dry weight of platelets %	Total lipid %
Protein	69	—	73	—
Total Lipid	16	—	17	—
Phospholipid	12	75	14	82
Free Cholesterol	2	13	2	12
Cholesterol Esters	2	12	1	6
Neutral Fat	0	0	0	0
Cephalin (% of total phospholipid)		68		85

[6] Ferguson, John H., *Am. J. Physiol.*, 1934, **108**, 670.

† The detailed procedure for the preparation of platelet samples and methods for chemical analyses are given in a preceding report.[7]

[7] Erickson, Betty N., Lee, Pearl, Williams, H. H., and Avrin, I., *J. Clin. Invest.* In press.

In contrast to their altered physical behavior, the blood platelets during menstruation exhibit no deficiency in their lipid composition. In fact, a greater proportion of the clot-aiding type of phospholipid, cephalin, appears to be present. No anomaly could be detected in the lipid composition of platelets from the blood of hemophiliacs; a discussion of other factors which must be investigated in relation to the physical behavior of blood platelets has been given.[7]

Summary. A definitely delayed platelet disintegration rate in menstruation is demonstrated by these studies. Hemophilia also shows similar changes. Lipid analysis of the platelets demonstrates no deficiency of cephalin in either menstruation or hemophilia.

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Time of Death of Lethal Homozygotes in the *T* (Brachyury) Series of the Mouse.*

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Dunn[1] reported the third lethal that was found in the *T* (Brachy) series in the house mouse. This lethal (t^1) was determined by Dunn in a tailless line, which had been isolated by Dobrovolskaia-Zavadskaia and Kobozieff.[2] Tailless mice of this line (29) are Tt^1. Results of matings of tailless mice of line 29 and tests of their descendants showed that both homozygous combinations TT and t^1t^1 are lethal, but the combination Tt^1 is viable.

In order to learn more about the lethal embryos t^1t^1, uteri of pregnant females were examined in different stages. The females used were all heterozygous normal-tailed mice ($+t^1$) and they were mated to heterozygous normal-tailed males ($+t^1$).

Table I shows the results of dissections of pregnant females at the age of 7, 8, 9, 10, and 11 days after fertilization. A total of 40 litters were dissected out, yielding 294 embryos. Of these, 275 were normal and 19 abnormal. Of the abnormal embryos, 13 were resorbed and 6 showed different kinds of abnormalities, but were not resorbed. If the t^1t^1 homozygotes died after implantation,

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1 Dunn, L. C., *Proc. Nat. Ac. Sc.*, 1937, **23**, 474.

2 Dobrovolskaia-Zavadskaia, N., and Kobozieff, N., *C. R. Soc. Biol.*, 1932, **110**, 782.