Effect of oral contraceptives on factor VIII clotting activity and factor VIII related antigen in normal women

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SYNOPSIS The factor VIII clotting activity (VIII_c) and factor VIII related antigen (VIII_{RAG}) were determined repeatedly in 24 pairs of age-matched normal women, one of each pair being on oral contraceptives. No significant differences in either parameter or in the VIII_c/VIII_{RAG} ratio were found between the two groups, although the mean factor VIII clotting activity and VIII_c/VIII_{RAG} ratios for women on oral contraceptives were very slightly higher than for those not on oral contraceptives.

The demonstration by Zimmerman *et al* (1971) that the ratio VIII_c/VIII_{RAG} is decreased in carriers of haemophilia has led to a more reliable method of identifying the carrier state than is possible when only factor VIII clotting activity is measured. Since many suspected carriers of haemophilia take oral contraceptives, it is important to determine whether the use of these drugs results in a change of the VIII_c/VIII_{RAG} ratio. Egeberg and Owren (1963) found that they led to an increase in the level of factor VIII clotting activity in normal women while Schiffman and Rapaport (1966) found an increase in factor VIII clotting activity levels in suspected carriers of haemophilia who were taking oral contraceptives. Their effect on factor VIII related antigen has not been previously reported. We present here the results of factor VIII clotting activity and factor VIII related antigen determinations on two groups of normal women, one on oral contraceptives and the other off them.

Materials and Methods

Blood samples were obtained from 24 normal female volunteers without a history of bleeding who were taking oral contraceptives and had been doing so for at least six months, and from 24 age-matched controls who had not taken oral contraceptives for at

least six months. Each pair was tested together on the same day in three successive weeks. The day of the menstrual cycle was recorded on each occasion that tests were carried out. Day 1 was the first non-pill day in the oral contraceptive group and the first day of bleeding for the controls.

The age distribution of the 24 pairs of women was as follows:

Number of Pairs	Age
5	20-25
5	25-30
4	30-35
5	35-40
5	40-45

COLLECTION OF BLOOD

Blood was collected, either at the place of work or at the home of the volunteer, by clean venepuncture into a plastic syringe and was immediately transferred to a polystyrene pot containing one-ninth volume of 3.8% trisodium citrate. After collection the sample was immediately taken to one of the participating laboratories where it was centrifuged at 3500 g for 20 minutes; the platelet-poor plasma was removed and half the specimen was taken to the other hospital laboratory. In each laboratory assays for factor VIII clotting activity were performed on the fresh sample, and an aliquot of each was frozen and stored at -30°C for subsequent determination of factor VIII related antigen.

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Factor VIII Clotting Assays

These were carried out by technicians who did not know whether the specimens they were testing were from women on or off oral contraceptives. Each specimen was tested by four different technicians (two at each hospital) within three hours of venepuncture. The standard used for all assays was the freeze-dried third British factor VIII plasma standard 72/244. The methods used at the respective laboratories were as follows:

Hospital for Sick Children:

One-stage method of Hardisty and Macpherson (1962)

Two-stage method of Denson (1966) (Diagen Kit)

Royal Free Hospital:

Two-stage method of Biggs *et al* (1955)

Factor VIII Related Antigen

The modification of the Laurell technique (1966) for quantitation of plasma proteins described by Zimmerman *et al* (1971) was carried out independently at both hospitals. The techniques for preparing the antiserum differed slightly between the two hospitals and were as follows:

At the Hospital for Sick Children the factor VIII antibody was raised in rabbits by repeated injection at multiple subcutaneous sites of factor VIII concentrate prepared by the method of van Mourik and Mochtar (1970). The antiserum was absorbed by incubation overnight at 37°C with an equal volume of platelet-poor plasma from a patient with severe von Willebrand's disease (factor VIII less than 1%) and was then heated at 60°C for one hour. After cooling, the mixture was absorbed for 10 minutes with tricalcium phosphate (10 mg/ml of antiserum) and then centrifuged at 3500 g for 10 minutes. The antiserum formed a precipitin line against normal, haemophilic, and afibrinogenaemic plasma and against serum, but not against plasma from a patient with severe von Willebrand's disease. It was used at a 1:200 dilution in the quantitative immunoassay procedure. A frozen plasma standard for factor VIII related antigen was prepared from a pool of 10 normal male donors between the ages of 20 and 50 years.

At the Royal Free Hospital the factor VIII antibody was raised in rabbits by repeated injection at multiple subcutaneous sites of normal cryoprecipitate and absorbed initially for four hours with 1/5 vol. supernatant plasma from normal cryoprecipitate and then repeatedly for four hours with 1/5 vol. platelet-poor plasma from the same severely

affected patient with von Willebrand's disease. The antiserum was absorbed twice with 1/10 vol. aluminium hydroxide suspension, centrifuged at 3500 g for 10 minutes at room temperature, and heated to 56°C for one hour (modified method of Kernoff *et al* (1974). The antiserum was used at a 1:80 dilution. A frozen plasma standard was prepared from a pool of 12 normal donors of both sexes between the ages of 20 and 50 years.

STATISTICAL ANALYSIS

The means and standard deviations were calculated for each of the four groups of results of factor VIII clotting assays (Hospital for Sick Children, one-stage and two-stage, Royal Free Hospital two-stage $\times$ 2), for factor VIII related antigen determinations in each laboratory, and for VIII_c/VIII_{RAg} ratios for women both on and off oral contraceptives.

Since the distribution for both factor VIII clotting activity and factor VIII related antigen was approximately log normal in type, log transformations were used for the analysis of these results. The values for VIII_c/VIII_{RAg} ratios followed a normal distribution and were analysed without transformation. Student's *t* test was used to compare the results of the two groups of women for each factor VIII clotting assay method and technician separately, each factor VIII related antigen determination, and the VIII_c/VIII_{RAg} ratios derived from each relevant pair of determinations.

Results

The results for both groups of patients are summarized in the Table. There was no significant difference between the two groups of women in either factor VIII clotting activity, factor VIII related antigen, or VIII_c/VIII_{RAg} ratio, although for each factor VIII clotting assay method the mean for the group of women taking oral contraceptives was slightly higher than the corresponding mean for the control group.

The results were also analysed separately for the five different age groups but showed no significant difference.

Individual analysis of the different factor VIII clotting assays showed a slightly wider variation in the one-stage assay although in the overall means there was no significant difference between the one- and two-stage assays.

A VIII_c/VIII_{RAg} ratio of less than 0.7 was obtained in eight women (four on oral contraceptives and four controls) when this was calculated on the basis of a single factor VIII clotting assay (by any of the three methods) and one immunoassay.

Test	Women on Oral Contraceptives		Women off Oral Contraceptives		P
	Mean	Mean $\pm$ 1 SD	Mean	Mean $\pm$ 1 SD	
Factor VIII clotting activity ¹					
One-stage HSC	94	67-133	91	65-129	0.60
Two-stage HSC	105	78-143	101	76-134	0.35
Two-stage RFH	101	76-134	96	73-126	0.30
Two-stage RFH	88	66-118	86	64-116	0.56
Factor VIII related antigen ¹					
HSC	93	60-144	93	63-138	0.96
RFH	83	56-123	82	57-120	0.89
Ratio VIIIc/VIII _{RAg}					
One-stage HSC	1.09	0.63-1.54	1.04	0.61-1.48	0.58
Two-stage HSC	1.18	0.80-1.56	1.12	0.81-1.43	0.30
Two-stage RFH	1.26	0.86-1.65	1.23	0.78-1.68	0.72
Two-stage RFH	1.09	0.83-1.35	1.09	0.76-1.42	0.96

Table Summary of results

¹log values used in calculation of FVIIIc and FVIII_{RAg} results. Raw data used for calculation of ratios.
HSC = Hospital for Sick Children; RFH = Royal Free Hospital

Discussion

Although the average factor VIII clotting activity of female carriers of the haemophilia gene is about half the average normal level, the wide variation in both normal women and carriers results in such a great overlap between the two groups that the carrier state can be forecast with confidence by this means alone only in a small minority of heterozygotes (Kerr *et al*, 1965). Zimmerman *et al* (1971) have shown, however, that the reliability of carrier detection can be substantially increased by relating factor VIII clotting activity of individual plasma samples tested to their factor VIII related antigen concentration, and although other investigators have failed to confirm the claim that as many as 90% of carriers can be detected in this way (Prentice and Forbes, 1974), there is no doubt that the VIII_c/VIII_{RAg} ratio forms a much better basis for carrier detection than factor VIII clotting activity alone. The need to determine the ratio on at least three occasions before reaching a conclusion is emphasized by the fact that eight of our normal women were found to have a ratio below 0.7 on a single occasion, although the final average ratio was normal in each case.

It is obviously important, therefore, to determine whether this proportionality between clotting and antigenic activity of factor VIII is disturbed by non-genetic influences, which might interfere with carrier detection. That oral contraceptives might have such an effect was suggested by Bouma *et al* (1973) and van Royen and ten Cate (1973), who showed that factor VIII related antigen increased more than factor VIII clotting activity during the last trimester of pregnancy. Our findings indicate, however, that the ratio is unaffected by oral contraceptives in normal women, and we therefore infer that this test

can be used with the same degree of reliability whether or not women are taking oral contraceptives.

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