Blood Coagulability and Oral Contraception*

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Thromboembolic episodes associated with the use of steroid oral contraceptives have been reported in England (*Brit. med. J.*, 1962) and are also known to have occurred in subjects who were on "enavid" therapy (norethynodrel plus ethynyl-oestradiol-3-methyl ether) in the United States. These latter deaths led to a study in this laboratory of the effect of long-term therapy with enavid on the activity of certain blood-clotting factors. During evaluation of the data Egeberg and Owren (1963) reported on the association between therapy with enavid and the increase of factor VIII and proconvertin in plasma. These authors did not report any change in the concentration of fibrinogen, which is contrary to our observation of a significant increase thereof associated with enavid therapy (Amundson and Pilgeram, 1964). This discrepancy may be due to the fact that their five subjects were on short-term therapy of 12 to 20 days, whereas our plasma donors had been on treatment with the steroid contraceptive for 5 to 18 months. This consideration is supported by the observation that oral contraception mimics the hormonal effects of pregnancy, in which there is found an increased concentration of fibrinogen (Dieckmann and Wegner, 1934).

The probability of these changes in coagulatory factors being related not only to enhanced coagulability of the blood but also to thrombosis is underscored by the fact that (a) the gravid state is frequently complicated by thrombosis, and (b) a marked increase occurs in the concentration of plasma fibrinogen in arteriosclerotic subjects who have recovered from myocardial infarcts and who are no longer on anticoagulant therapy (Pilgeram, 1961). Since we had found a highly significant increase in the activity of plasma antithrombin in the ageing arteriosclerotic subject (Pilgeram, Schram, and Loegering, 1963), it seemed important to establish whether the activity of this factor was also enhanced by use of enavid at recommended contraceptive dosage. The results show a pronounced increase in activity of antithrombin associated with the steroid therapy.

EXPERIMENTAL

Human prothrombin was prepared according to a modified procedure of Seegers (1952) for isolation of bovine prothrombin. The final step of isoelectric fractionation was omitted and all human reagents were employed. Human factor V was prepared according to the chromatographic procedure of Cox, Lanchantin, and Ware (1956). Thromboplastin was prepared from human lung according to the procedure outlined by Ware and Seegers (1949) for bovine material. Human platelet extract was prepared according to the method outlined by Spurling and King (1954). Preparation of fibrinogen was patterned after the procedure of Ware, Guest, and Seegers (1947). Normal plasma samples were obtained from nine young women aged 20 to 26 years (mean age 22.4 years). Nine other female subjects aged 20 to 30 years (mean age 24.7 years) received 5 mg. of norethynodrel plus 0.075 mg. of ethynyl-oestradiol-3-methyl

ether¹ daily for at least 11 days prior to donation of the plasma sample. All of the enavid subjects had been on the cyclical birth-control therapy for more than five months preceding the analysis. Plasma was drawn after an overnight fast of 8 to 12 hours. Anticoagulant consisted of 1 ml. of dry (shell-lupophilized) potassium oxalate (0.1 M) per 10 ml. of blood.

Paired duplicate or triplicate assays on a normal and experimental plasma sample were conducted simultaneously under identical conditions immediately after withdrawal of the sample. Since the study was of several months' duration, this approach obviated variation incurred by technical variables and biological reagents with differing levels of activity. Each member of the above pair was matched for age and for the period of the menstrual cycle. To defibrinate plasma, 0.1 ml. of thrombin (100 μ /ml.) was added to 0.9 ml. of plasma. After 20 minutes at 22° C., the fibrin clot was removed and fluid exuded with a hardwood applicator stick (medical applicators, Hardwood Products Co., Guilford, Maine). The adequacy of defibrination is important. Fibrin adsorbs thrombin; hence an elevated level of plasma fibrinogen in one of the groups could influence the assay of antithrombin. The adequacy of defibrination was demonstrated in each experiment by noting the absence of any clot or fibrin strands in the antithrombin assay in which diluted plasma was incubated with thrombin for two hours. This method of defibrination has been shown to destroy antithrombin (Pilgeram *et al.*, 1963); however, it was the preferred approach in order to obviate any adverse or variable effect resulting from defibrination at 53° C. for 3 minutes. It is not possible for defibrination with thrombin to have caused a variable in the activity of antithrombin, since each plasma specimen was reacted with equivalent levels of thrombin. Antithrombin was assayed according to the methods previously used in this laboratory (Pilgeram *et al.*, 1963).

RESULTS

Norethynodrel 5 mg. and ethynyl-oestradiol-3-methyl ether 0.075 mg. daily induced a 24.1% ($P < 0.012$) increase in plasma antithrombin when tested by the clotting-time method. The same subjects displayed a 20.7% ($P < 0.007$) increase in antithrombin activity when assayed by the tosylarginine methyl ester (TAME) procedure (see Table). Variation in the activity levels measured by either method are due in part to biological variability and in part to variation of specific activity in different thrombin preparations. This latter variability was controlled in the assay by always running a control and an experimental plasma at the same time with the same thrombin preparation. Routine reproducibility of either of the two methods appeared

Effect of Norethynodrel and Ethynyl-oestradiol-3-Methyl Ether on the Activity of Human Plasma Antithrombin

Expt. No.	Clotting-time (Thrombin Units/ml. Plasma)			TAME (μ M/ml. Plasma)		
	Control	Steroid Therapy	Increase (%)	Control	Steroid Therapy	Increase (%)
1	852	1,062	24.6	—	—	—
2	984	1,086	10.4	476	488	2.5
3	726	1,176	61.9	—	—	—
4	—	—	—	275	348	26.5
5	1,132	1,368	15.7	244	288	18.0
6	1,020	1,296	27.0	305	448	46.9
7	1,296	1,524	17.6	380	438	15.3
8	1,530	1,710	11.8	414	486	17.4
9	—	—	—	203	240	18.2
Mean increase %			24.1 $P < 0.012$	20.7 $P < 0.007$		

The confidence level is calculated by the equation (Pilgeram *et al.*, 1963), which permits determining the test statistic in paired experiments where the absolute level of the pair may change from one experiment to another but where the percentage change remains substantially constant.

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¹ Sold under the trade name "enavid" in the U.K. and "enovid" in the U.S.A.

acceptable with a variability of less than 1% in multiple assays on a given sample.

DISCUSSION

The significance of the effect of these steroids on plasma antithrombin is not clear. The elevation in the concentration of plasma antithrombin may be due to (a) an unused build-up of antithrombin due to deficient formation of thrombin or to (b) an enhanced biosynthetic feed-back response to increased production of thrombin. Within our current state of knowledge, a case may be made for both concepts (Pilgeram *et al.*, 1963). These pathways are based on the assumption of the presence of a continuous haemostasis or formation of thrombin. If the change in antithrombin is a primary response to thrombin, then it is possible that one or both enavid steroids induce a stimulatory effect on the plasma and tissue-thromboplastin-generating system, since it is this system which most probably would be responsible for controlling the activity level of thrombin. An alternative to this concept is that the steroid directly affects the biosynthesis or utilization of antithrombin and fibrinogen.

SUMMARY

Daily ingestion of the oral contraceptive "enavid" (norethynodrel 5 mg. plus ethinyloestradiol-3-methyl ether 0.075 mg.)

will increase the activity of plasma antithrombin by 24.1% ($P < 0.012$) when assayed by the clotting-time method. A 20.7% ($P < 0.007$) increase is observed when the activity of antithrombin is measured by the TAME procedure. A correlation is noted between this effect and the change in the activity of antithrombin in the ageing subject who has recovered from a thrombotic attack and who is not on anticoagulant therapy.

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Medical Memoranda

Prolapsed Intervertebral Disk in a Case of Ankylosing Spondylitis

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Those who treat low backache must be aware of the difficulties occasionally arising in differential diagnosis between chronic lumbar disk lesions and ankylosing spondylitis. Recent reports do not discuss the possibility of coincidence, though many describe cases of ankylosing spondylitis confused with disk lesions and even subjected to exploration (Key, 1951). Key, however, stressed that in one patient "the acute sciatica was relieved by operation and it is probable that he suffered from both conditions." Such a case is reported here.

CASE REPORT

While serving in the R.A.F. in 1942, the patient, then aged 28, developed low back pain. Ankylosing spondylitis was diagnosed within three months of the onset of symptoms and he was discharged from the Service. In 1949 he was seen by one of us (J. K. S.) and he derived great benefit from a course of x-ray therapy. Thereafter, working as a supervisor in a paint factory, he experienced only mild discomfort after severe activity.

In September 1959 he was involved in a minor motor accident and his back was jerked. Half an hour later his back was very painful and stiff, and after a fortnight he developed right sciatica for the first time. Conservative treatment was pursued for eight months without lasting benefit. In 1960 he was admitted to the neurological department of the Royal Victoria Infirmary under the care of Dr. Henry Miller and was later transferred to the orthopaedic department.

Findings.—There were a dorsal kyphosis and a lumbar scoliosis to the left. Movement in his lower back was limited, and straight-

leg raising was 40 degrees on both sides. The right ankle-jerk was absent; the right calf was wasted, and there was hypoaesthesia on the outer aspect of the right leg. X-ray examination showed characteristic changes of ankylosing spondylitis, most pronounced in both sacro-iliac joints (Fig. 1). There were changes also at the dorso-lumbar region of the spine, but lumbar puncture revealed no abnormality and the C.S.F. was normal. Myelography showed incomplete filling of the right fifth lumbar root sleeve (Fig. 2). Prolapsed intervertebral disk was diagnosed.

FIG. 1.—Antero-posterior radiograph of the sacro-iliac joints and lumbar spine. Involvement of sacro-iliac joints and posterior articulations of the lumbar spine is demonstrated.