thrombin, platelets, ac-globulin and fibrinogen clots faster than intact blood, is an indication of the effectiveness of inhibitors in blocking or offsetting changes in these coagulation factors. Moreover, since the coagulation of hemophilic blood or plasma is greatly shortened by dilution, it is difficult to understand how a deficiency of a plasma constituent can be responsible for the defect in this disorder. Dilution would naturally tend to accentuate the deficiency and thereby further delay coagulation.

Summary. Crude and purified cephalin and antithromboplastin extracted from brain tissue yield characteristic activity curves when tested at different concentrations, on stable citrated normal human plasma. Moderate dilution accelerates the coagulation of normal and hemophilic blood and plasma and delays that of posthemorrhagic blood and plasma. Excepting fraction V, all Cohn's plasma fractions have coagulant action; only fractions IV-1 and IV-4 display a biphasic curve of activity, an indication that they are mixtures of coagulants and anticoagulants.

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Does Administration of Diethylstilbestrol to Pregnant Women Result in Increased Output of Urinary Pregnanediol?*

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In 1946 there appeared in the literature the interesting observation that the oral administration of diethylstilbestrol to a pregnant diabetic woman resulted in an increased excretion of urinary pregnanediol as measured by the Venning method.[1] This finding was interpreted by the authors as an index of increased production of steroids by the placenta brought about by estrogenic stimulation. They suggested the oral administration of diethylstilbestrol in the prevention and treatment of the accidents of late pregnancy such as the toxemias, premature fetal death and diabetic complications associated with gestation.

In an earlier report[2] we were unable to demonstrate increased pregnanediol excretion in patients receiving large amounts of diethylstilbestrol early in pregnancy. During the past 2 years we have studied a large group of patients who presented a variety of pregnancy complications in early and late gestation. Large amounts of diethylstilbestrol (5 to 200 mg daily) were administered to these women and bi- and tri-weekly urinary pregnanediol determinations were made by a method described by us in a previous communication.[3] No obvious increase in free pregnanediol was apparent in any of our studies. The results of these observations are in the process of publication.

The apparent discrepancy in the results obtained from the administration of diethylstilbestrol to pregnant women by the Smiths and in our own laboratory led us to seek an explanation. It has been reported in numerous publications[4-7] that in the metabolism of diethylstilbestrol it is conjugated and ultimately excreted as a glucuronide. The Venning

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4 Zondek, B., and Sulman, F., *Nature*, 1939, **144**, 596.

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TABLE I.
Simultaneous Urinary Pregnanediol Determinations by the Venning Method and the Free Pregnanediol Method in Normal Pregnant Patients and in Pregnant Patients Who Were Taking Diethylstilbestrol.

No.	Free preg.,* mg/24 hr	Administered diethylstilbestrol, mg	NaPG* calculated as preg., mg/24 hr
1	11.93	0	7.91
2	19.72	0	15.54
3	19.68	0	18.00
4	28.99	0	28.75
5	35.40	0	33.80
6	48.80	0	48.70
7	11.53	25	13.98
8	41.90	50	60.44
9	11.64	50	35.26
10	36.85	100	69.15
11	28.73	115	75.42
12	20.77	125	60.10
13	31.05	125	83.55
14	51.46	125	96.13

* These are uncorrected results. No attempt has been made to account for losses during extraction.

method for the determination of urinary pregnanediol measures the glucuronide titer of the urine. Thus, other glucuronides than pregnanediol are included in the results obtained. The method that has been in use in our laboratory determines the amount of free pregnanediol after acid hydrolysis.[3] The following experiments were carried out to compare the results obtained by these two methods in normal pregnant women who were receiving no medication and in those to whom diethyl stilbestrol in varying amounts was administered.

Methods and results. A series of 24 hour urine collections from normal women during various periods of gestation were analyzed simultaneously by the Venning method and by the method for free pregnanediol used in our laboratory. There was an extremely close correlation in the results obtained by the two procedures (Table I). The figures tabulated are uncorrected since it seemed to us that some loss of pregnanediol would occur in both methods.

Simultaneous pregnanediol determinations by both methods were made in a second group of pregnant patients to whom varying amounts (25 to 125 mg) of diethylstilbestrol were administered daily. The Venning method yielded substantially higher values than the method used in our laboratory. Furthermore, the increase obtained by the Venning method over that obtained by the free pregnanediol procedure was roughly in proportion to the amount of diethylstilbestrol administered to the patient.

A small group of patients in various stages of pregnancy under observation in the hospital was used for the following experiment. The urine was collected for several 24 hour periods and urinary pregnanediol levels were determined by both methods. The patients then received a single oral dose of 200 mg of diethylstilbestrol and urine collections continued. These, too, were analyzed for pregnanediol. The results of the determinations prior to the administration of diethylstilbestrol were comparable in both procedures. However, there was a marked increase in the amount obtained by the Venning method following the administration of the estrogen. (Fig. 1) Furthermore, in most cases this increase occurred during the 24 hour period following the ingestion of the diethylstilbestrol but in one patient there was a short delay so that the increased amount did not appear until

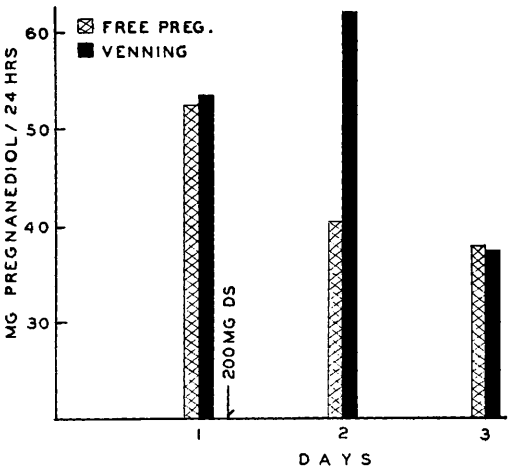

FIG. 1.
Simultaneous urinary pregnanediol determinations by the Venning method and the free pregnanediol method in a normal pregnant patient before and after the administration of diethylstilbestrol.

[7] Smith, A. E. W., and Williams, P. C., *Biochem. J.*, 1948, **42**, 253.

[8] Venning, E. H., *J. Biol. Chem.*, 1937, **119**, 473.

the second 24 hour urine collection. This patient had serious kidney damage which may have accounted for the delay in excretion. No attempt was made to determine the amount of diethylstilbestrol excreted in the feces.

Finally, the material obtained from the Venning procedures was assayed qualitatively for estrogenic activity using the castrated female guinea pig as the test animal. Opening of the vaginal canal by dissolution of the vaginal membrane was taken as the index of estrogenic activity. It was found that only those samples which showed an increased pregnanediol titer with the Venning method over the free pregnanediol method exhibited estrogenic potency during the period of observation.

Summary and conclusions. When diethylstilbestrol is administered to a woman during pregnancy, much of this material appears in the urine as a glucuronide. In the Venning method for the assay of urinary pregnanediol, all of the glucuronides are included in the final determination. Thus diethylstilbestrol glucuronide as well as pregnanediol glucuronide appears in the result obtained. The apparent rise in the urinary pregnanediol values obtained by the Venning method may be due to the ingested diethylstilbestrol which is conjugated and eliminated in the urine. The figures do not indicate an increased production of progesterone and resultant increased output of urinary pregnanediol.

Increasing amounts of diethylstilbestrol are being used in the treatment of pregnancy complications. In most instances the theory behind this therapeutic measure is that this estrogen stimulates steroid production. If urinary pregnanediol is to be used as a measure of increased steroid metabolism the value of diethylstilbestrol is open to question. It is possible that diethylstilbestrol exerts a favorable influence on placental circulation or on early placental development. However, there is no evidence that it results in an increased production of progesterone if urinary pregnanediol is to be regarded as an index of progesterone metabolism.

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Age Factor in Estrogen-Induced Breast Cancers of Mice.*

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In male mice of strains C3H and D, the age at the beginning of the treatment was one of the factors which determined the susceptibility of these animals to estrogen-induced breast cancers[1] and leukemias.[2-4] The breast cancer rate was higher in mice injected with estrogenic hormone before or about the onset of sexual maturity than in those receiving the hormone from the age of 4 to 6 months on. In female mice similarly treated, this age effect was much less conspicuous or lacking. According to Loeb,[1b] these findings suggest the following interpretation: (1) In younger mice, the breast tissue itself might be more susceptible to growth stimulation than in older animals. If this is the case, however, the lack of an age effect in females would be difficult to explain. Still, such an age factor might be present in female mice also, but it might be obscured or neutralized by the periodic stimulation of the mammary gland by the intrinsic estrogenic hormone produced in

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1 (a) Loeb, L., *Harvey Lectures*, 1941, **36**, 228; (b) Loeb, L., *Biol. Symposia*, 1945, **11**, 197; (c) Loeb, L., Suntzeff, V., Burns, E. L., and Schenken, I. R., *Arch. Pathol.*, 1944, **38**, 52.

2 Murphy, J. B., *Cancer Res.*, 1944, **4**, 622.

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