counted for on the basis of the following characteristics of D_2O: (1) extracellular as well as intracellular distribution, (2) unusually high diffusibility through capillary walls, and (3) escape of a considerable portion from metabolic binding.

The mechanism for the frequently observed increasing per cent net transport during the early period of the downslope of l-methionine is not apparent. Possible explanations might be that metabolic incorporation is not as efficient when peak concentrations are present, or that the later downslope represents plasma moving at lesser velocity permitting more complete transcapillary exchange.

Summary and conclusions. 1) In the forearm the maximum transcapillary loss of S^{35}-labeled l-methionine averaged 47.6 ± 7.3%. Methionine exhibited a pattern of transcapillary net exchange which is consistent with intracellular binding. A high rate of net loss was observed for a much longer period than with thiocyanate ion and the equilibrium time was delayed considerably. Net return to the circulation was small compared to thiocyanate during the period of observation. 2) On the basis of these and other observations it is suggested that 3 distinct classes of substances have characteristic patterns of transcapillary net exchange which may be distinguished with the present technic. These substances are (a) primarily extracellular ions, (b) primarily intracellular diffusible substances, and (c) highly diffusible molecules which are distributed throughout both compartments (body water).

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Changes in Plasma Prothrombin, Ac-Globulin, and Antithrombin Concentration Following Intravenous Administration of Estrogens.* (22864)

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Recent observations made in clinics indicate that estrogens given intravenously are of therapeutic value for control of spontaneous hemorrhage, such as epistaxis, excessive postoperative bleeding from tonsillectomies and, on occasion, from urological surgical procedures(1,2). Moderate success has also been reported for this therapy in spontaneous gastrointestinal bleeding. These uses are of more recent application than for control of uterine bleeding for which estrogens have had long popularity. The studies here reported are regarded as a preliminary survey designed to determine what changes may be produced in concentrations of blood coagulation factors following intravenous injection of estrogens. Previous investigators reported alterations of clotting factors coincident with physiological changes in levels of estrogenic hormones, as well as a rise in heparin like antithrombinic activity with administration of synthetic estrogen(3). For a brief period ovarian extracts were given extensive trials for control of bleeding tendency in hemophilia(4), but this did not become established practice(5). During pregnancy there is elevation of prothrombin level(13) near term as well as in

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serum prothrombin conversion accelerator (6) and fibrinogen(7). During the menstrual cycle, platelet numbers increase at ovulation and fall at menses(8). With administration of 20 mg of estrogens intravenously prompt cessation of hemorrhage has been noted(2). Occasionally bleeding commenced again after 4 to 5 hours but with repetition, bleeding stopped. Toxic effects or side reactions have not been noted for these short term administrations although prolonged use might lead to endocrinological disturbances.

Material and methods. The estrogenic preparation, Premarin, is described as amorphous complex containing sulphates of steroids occurring in pregnant mares urine. The preparation also contains small amounts of nonsteroid phenol sulphates. The preparation of laboratory reagents for investigation of blood clotting mechanisms has been described previously as follows: Purified prothrombin(9), purified thrombin(10), bovine platelet suspension(11), assays of prothrombin(12), thrombin(14), fibrinogen(15), Ac-globulin(16), antithrombin(17), autoprothrombin I(18), platelet cofactor I(19), and platelet cofactor II(20).

Results. Prior to intravenous injection of Premarin, it was used in the test tube as an added or substituted factor in analyses mentioned above without significant change being noted in any of the assays.

Dogs of both sexes were anesthetized with Nembutal. A control blood specimen was drawn using 3.2% sodium citrate as anticoagulant. Ten mg of estrogen preparation was then given intravenously and blood specimens were drawn at intervals using a 2-syringe technic to avoid contamination of specimens with tissue thromboplastin. Plasma was obtained by centrifugation. A sample of blood was also drawn to obtain serum. Usually analyses were then done at once or the specimens were frozen and stored in deep freeze for analysis the next day. Larger doses of estrogens were given to 2 dogs and there was a proportionately greater change in measured values in the same direction as with the small dose. Nine male and 3 female dogs were studied. All indications from these animals confirm the selected example recorded in Table I.

TABLE I. Changes Observed in Coagulation Factors after Intravenous Injection of Estrogens.

Time, min.	Prothrombin, units/ml	Ac-globulin, units/ml	Antithrombin*
Control	134	32	790
	(Premarin inj. of 10 mg given intrav.)		
15	162	36	960
30	185	90	880
55	190	70	860
130	152	86	890
150	175	100	760
240	145	72	700

* Expressed as units of thrombin remaining after incubation for 2 hr with a substrate of about 1400 units of thrombin/ml.

Discussion. Elevation of prothrombin values as well as depression of antithrombin content of plasma, increases the amount of potential thrombin available and also tends to make it more effective. This allows thrombin to manifest its multiple functions in the clotting process to a greater degree, particularly in the presence of increased amounts of Ac-globulin.

The values for Ac-globulin rose markedly. In the dog, these values are normally high; in the human, the normal values are only 10 to 15 units/ml. The latter figure is one of the lowest of all species tested(21), so that depression of a few units may be of importance in the etiology of hemorrhage. Conversely, an elevation might be of importance for the prevention or control of bleeding. Should human plasma have a rise in Ac-globulin values following estrogen administration proportional to that in the dog, this would be of unusual advantage to man.

Summary. Solutions of estrogenic steroids were administered intravenously to dogs. There was a sharp rise in the plasma Ac-globulin and prothrombin concentration. These changes began within the first 15 minutes, reached a peak in about 1 and ½ hours. In about 3 or 4 hours normal concentrations were again found. A small decrease in antithrombin activity of plasma was found. Theoretically all these changes tend to enhance coagulability of the blood.

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Increase in Tolerated Dose of Isoniazid in Mice by Use of Cycloserine. (22865)

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Isoniazid continues to be in widespread use in the therapy of human tuberculosis. The toxicity of the drug is a limiting factor in dosage. In animal experiments with this compound, it has been found possible to increase the tolerated dose by the use of suitable detoxifying agents(1). Such agents were also employed to investigate the possible detoxication of the new antibiotic cycloserine,* D-4-amino-3-isoxazolidone, of current interest in the treatment of human tuberculosis(2-4). Unexpectedly, it was observed that simultaneous administration of isoniazid (INH) and cycloserine (CS) resulted in a slight reduction of the hyperactivity induced in mice by the CS, and marked reduction in the percentage mortality in mice given larger, otherwise lethal, doses of INH. This latter observation prompted the present detoxication study with these two chemicals.

Methods. Control toxicity tests of each chemical by gavage in DBA mice showed that (a) INH permitted 45% survival with a 5 mg dose and no survival with 6 mg (250 and 300 mg/kg respectively)(1); and (b) CS was tolerated by 6 of 7 mice each given 250 mg, but was 100% lethal with 300 mg (12.5 and 15 g/kg respectively). CS in amounts of 25 mg or more caused hyperactivity evidenced by continuous circling in the jar beginning about 30 minutes after administration of the chemical.

Results. Tolerance limits after a single dose of mixed chemicals. The effect of a single oral dose of mixtures of various concentrations of CS and INH on survival time of

* Kindly supplied as seromycin by Eli Lilly and Co., Indianapolis, Ind.