far as we know now can be circumvented only by employing relatively high dilutions of such materials, or perhaps, in certain instances, by the simultaneous intravenous injection of heparin. In the preparation of tissue cultures where chicken plasma is used, mammalian tissues or tissue extracts cannot be relied upon to induce the formation of a firm coagulum. The results of experiments designed to determine the anaphylactogenic capacity of various tissues or organs might be misinterpreted if the investigator were unaware of the rapid death which may follow the intravenous injection of tissue suspensions from certain related classes of animals and careful post mortem examinations were not made.

Conclusion. The toxic effect *in vivo* of certain tissue derivatives exhibits biologic specificity. This specificity appears to parallel that of thromboplastin on the clotting of blood plasma *in vitro.*

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The Preparation and Biological Activity of Alpha Estradiol-Sensitized Collodion Particles.* (17942)

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(Introduced by D. H. Sprunt)

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In the course of studies on the antigenic properties of crystalline alpha estradiol† (in combination with protein substances) we included collodion particle agglutination(1) as one of the tests for the presence of serum antibody. This serological procedure has proved to be a sensitive and reliable method of antibody detection and titration in our laboratory(2), as well as in others(3,4,5). The usual method of sensitization of collodion particles consists of incubation of a mixture of particles and antigen in optimal proportions. Non-specific absorption of antigen occurs, and the sensitized particles may then be combined with antisera in proper dilutions. This method of sensitization was not successful when aqueous suspensions of alpha estradiol were incubated with the collodion particles. We were not able to overcome the insolubility of the steroid substance in the aqueous medium. However, sensitization of collodion particles has been accomplished by incorporation of the steroid in and on the particle at the time of preparation.

Preparation of sensitized particles. Collodion U. S. P. Merck was treated in the manner recommended by Cannon and Marshall (1). Either a 5 or 10% acetone solution of the dried collodion constituted the stock solution to be used as a source of particles. Arbitrary amounts of crystalline alpha estradiol were dissolved in 100 ml quantities of the stock solution. In most instances we added sufficient alpha estradiol to give 100 mg per 100 ml of the collodion solution. The particles were then prepared and washed in the usual manner. The alpha estradiol content of the final particle suspension was calculated by lyophilizing quantities of 1-5 ml, weighing of the dried material and comparison

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TABLE I.
Biological Activity of Alpha Estradiol-Sensitized Collodion Particles.

Animal	Dose, ml	Lot	Calculated concentration of alpha estradiol, γ	Duration of cornified vaginal smear
Spayed mice	.1	2	1	9 days
	.1	3	1	9 "
	.1	1	1.6	27 "
	.2	1	3.2	45 "*
	.3	1	4.8	45 "*
Spayed rats	1.	1 (diluted)	1	5 "
	1.	2 "	1	5 "
	1.	3 "	1	5 "
	1.	3 "	5	15 "
	1.	2	10	30 days to date†
Weanling rats	1.	1	10	‡

Spayed mice gave no reaction to non-sensitized collodion particles.
* Duration of experiment.
† Cornified smear persists.
‡ Vaginal diaphragm opened in 48 hr, vaginal smear cornified through test period, 40 days.

with the collodion content of the original solution.

Animal tests. Biological evidence that the collodion particles carried the alpha estradiol was furnished by introduction of graded amounts of particle suspension into spayed mice and rats and into weanling rats. Table I presents the results of these tests. The spayed animals exhibited prompt evidence of estrogenic stimulation as indicated by the predominance of cornified cells in the vaginal smears. Within 48 hours the vaginal diaphragm of the weanling rats had opened and the vaginal smear showed only cornified cells. Moreover, the state of estrogenic stimulation following single injections in both species of animals persisted for substantially longer periods than those observed by us with the use of similar doses in oil or aqueous mediums. The periods of stimulation were related to the quantity of sensitized particles introduced. With the doses estimated to contain 10 gamma the animals have continued to show the effects of the hormone for periods of 4-6 weeks.

Apparently the alpha estradiol-sensitized particles provide an *in vivo* depot from which the estrogen is slowly eluted. It is believed that this demonstration that collodion particles may act as carriers of estrogens through incorporation of the steroid into and on the surface of the particles may offer opportunities for broad use. Detailed studies on this problem are in progress. These studies will include titrations of biological effects, histological examinations of the *in vivo* reservoir and use of the spectrophotometer for more exact determination of the alpha estradiol content of the collodion particle preparation.

Furthermore it is believed that such a method of sensitization of collodion particles may have practical use in study of other steroid "antigens" which include steroid-protein conjugates and tissue extracts. These data, along with those from use of the alpha estradiol-sensitized particles will form a separate report.

We should also like to emphasize that this method of sensitization of particles need not be limited to steroid substances. It should be possible to use collodion particles for the local deposit of various therapeutic reagents which owe their maximum effect to maintenance of adequate concentrations over a prolonged period.

Summary. Crystalline alpha estradiol may be dissolved in acetone solutions of collodion and the steroid incorporated in or on the collodion particles, prepared in the usual manner. Such particles are biologically active as shown by the onset of a cornified vaginal smear in spayed or immature mice and rats. Furthermore, the hormone stimulation appears to persist for prolonged periods, presumably due to the slow elution of the alpha

estradiol from the collodion particle.

Addendum. The alpha estradiol-sensitized collodion particles were active after a storage period of four and one half months in the refrigerator. This was established by the onset of cornified cells in spayed rats.

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Preparation of a Water Emulsion of α-Tocopherylhydroquinone Suitable for Intravenous Administration in Animals.* (17943)

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The recent observation that α-tocopherylhydroquinone† has vitamin E activity(1) made it desirable to have a preparation that is suitable for intravenous administration. The difficulty of preparing the tocopherols and those of their esters which are oils, in a form suitable for enzyme studies and for bioassay by parenteral administration is well known. These difficulties are increased in the case of α-tocopherylhydroquinone, because of the ease with which this substance is oxidized to tocopherylquinone. Ames(2) recently suggested the use of bovine serum as a menstruum for tocopherol in investigations on enzyme systems, and Sobel and co-workers (3,3a) have used aqueous dispersions of certain of the fat soluble vitamins. Since vitamin K can form a stable colloidal solution when added to sugar solutions(4) several sugars were investigated for their possible solubilizing effect on tocopherylhydroquinone. The 2 solvents, ethanol and propylene glycol have been employed in bioassays of tocopherylhydroquinone in this laboratory in the form either of a 100% propylene glycol solution, or of a 10% ethanol - 90% propylene glycol mixture. Propylene glycol produced traumatic effects on the blood vessels when injected intravenously daily. However, since these solvents are suitable for the chemical preparation of the α-tocopherylhydroquinone, both were investigated for their possible utilization in a satisfactory water emulsion. It was hoped that emulsions containing relatively low concentrations of these solvents might be free from the objectionable properties of the solvents themselves. The emulsions were not sterilized; this lack of sterilization appeared to offer no objection to their use in rabbits. However, similar employment in man should not be attempted.

Methods. Preparation of tocopherylhydroquinone. Tocopherylhydroquinone was prepared by hydrogenation of tocopherylquinone in either ethanol or propylene glycol, by the method previously described(1). The amounts of tocopherylquinone were such that the final solution contained 200 mg tocopherylhydroquinone per cc of ethanol, or 100 mg per cc of propylene glycol. The tocopherylhydroquinone was assayed colorimetrically by a modification of the Emmerie-Engel reaction(1).

Preparation of emulsions. Usually 1 cc of the solution was mixed with 9 cc of water in glass stoppered test tubes by underlaying

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