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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† The α-tocopherylhydroquinone used in these studies was prepared from α-tocopherol kindly supplied by Merck & Co., Rahway, N. J.

1. Mackenzie, Julia B., Rosenkrantz, H., Ulick, S., and Milhorat, A. T., *J. Biol. Chem.*, 1950, v183, 655.

2. Ames, S. R., and Risley, H. A., *Ann. New York Acad. Sci.*, 1949, v52, 149.

3. Sobel, A. E., Rossberg, A., Geduldig, R., Engel, E., West, M., and Kramer, B., *Fed. Proc.*, 1949, v8, 253.

3a. Sobel, A. E., Sherman, M., Lichtblau, J., Snow, S., and Kramer, B., *J. Nutr.*, 1948, v35, 225.

4. Frank, H. A., Hurwitz, A., and Seligman, A. M., *N. E. J. Med.*, 1939, v221, 975.

the water with the tocopherylhydroquinone solution and then shaking the tube for a few seconds. The amounts of tocopherylhydroquinone were determined at various intervals after formation of the emulsion. These determinations indicated both the rate of oxidation of the tocopherylhydroquinone and the stability of the emulsion.

Observations and discussion. Ethanol-water emulsions proved unsatisfactory, and attempts to stabilize them with sugars were unsuccessful since less than 10% of the tocopherylhydroquinone could be accounted for in the emulsions. Isotonic saline, likewise, was unsatisfactory. The oxidation of tocopherylhydroquinone proceeds more slowly in propylene glycol than in ethanol. Propylene glycol-water emulsions prepared as outlined above contained from 80% to 90% of the tocopherylhydroquinone that had been added. The remainder presumably was present in the white crust on the surface of the emulsion. After periods of about 48 hours, 75% of the added tocopherylhydroquinone could still be accounted for in the emulsion. The emulsion had a white, milky appearance and examination under the microscope revealed a homogenous arrangement of the globules. Filtration through No. 1 Whatman paper or centrifugation for periods up to ½ hour did not disturb the emulsion significantly. After the emulsion had stood at room temperature for more than 24 hours, oxidation of the tocopherylhydroquinone was evident by the appearance of the yellow color characteristic of the quinone. The rates of oxidation of tocopherylhydroquinone in the emulsion and in propylene glycol are compared in Fig. 1. Initial concentrations of the hydroquinone in the water emulsion and in propylene glycol were 6.81 mg/cc and 5.41 mg/cc, respectively. The preparations were kept at refrigerator temperatures except for the intervals when the concentrations of tocopherylhydroquinone were being determined. It can be seen from Fig. 1 that the rates of oxidation of tocopherylhydroquinone are practically identical in both systems. At refrigerator temperatures, only slight oxidation occurred during the first 3 days and the emulsion still contained 50% or more of the added tocopherylhydroquinone after a period of one week.

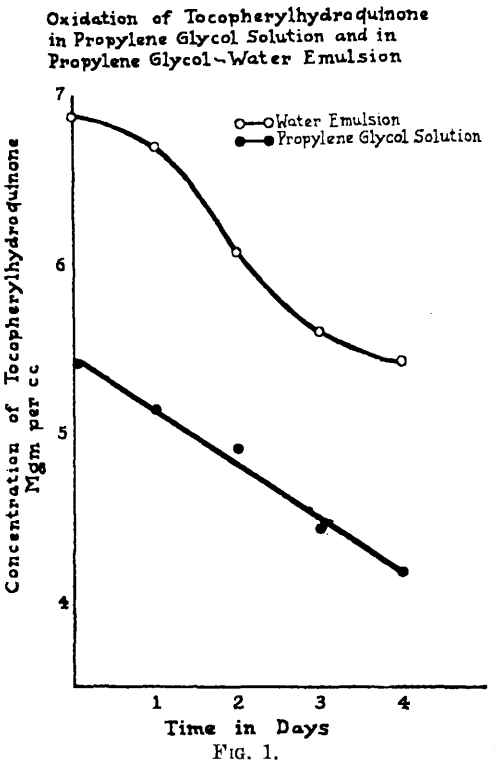

FIG. 1.

In order to ascertain if sugars could function as protective agents, emulsions composed of solutions of different sugars and tocopherylhydroquinone in propylene glycol were compared. The results are tabulated in Table I. Although the amounts of tocopherylhydroquinone that could be accounted for at zero time varied, these variations may not be significant and probably were the result of slight differences in the conditions permitting oxidation in the samples. However, there appeared to be no definite advantage in substituting solutions of sugars for distilled water in these emulsions.

Alterations in the concentration of either the tocopherylhydroquinone or the propylene glycol had adverse effects on the stability of the emulsion; maximal stability was achieved when the concentrations mentioned above were employed; namely, about 80 mg tocopherylhydroquinone in each 100 cc of a 10% propylene glycol-90% water emulsion. Tocopherol itself formed unsatisfactory propy-

TABLE I.
Effect of Certain Sugars and Other Substances on Formation and Stability of Propylene Glycol-Water Emulsion Containing Tocopherylhydroquinone.*

		THQ in emulsion†					
		At 0 time		At 24 hr		At 48 hr	
Substance added to water	THQ added to system mg/cc total vol.	mg/cc	% of added THQ	mg/cc	% of THQ at 0 time	mg/cc	% of THQ at 0 time
0‡	7.6	6.1	81	5.9	97	5.5	90
Inositol	7.6	6.5	86	5.9	91	5.4	83
Sorbitol	7.6	6.3	83	5.8	92	5.6	89
Arabinose	8.0	6.3	79	5.8	92		
Mannose	8.0	6.2	78	5.6	91	5.5	89
Galactose	8.0	6.2	78	5.7	92	5.0	81
Peptone	8.0	6.6	82	6.3	95	5.8	88
Glycine	8.0	6.4	80	6.0	94	5.3	83

* 1 cc propylene glycol containing tocopherylhydroquinone was added to 9 cc water or water containing the sugars or other substances as 1% solution.
† For purposes of brevity, tocopherylhydroquinone is designated as THQ.
‡ Control, water only.

TABLE II.
Effect of Salts on Propylene Glycol-Water Emulsion Containing Tocopherylhydroquinone.*

		THQ in emulsion†					
		At 0 time		At 1 hr		At 24 hr	
Salt	mg salt added	mg/cc	% of added THQ	mg/cc	% of THQ at 0 time	mg/cc	% of THQ at 0 time
	0‡	5.81	85	5.25	90	4.83	83.5
NaCl	9	5.72	84	5.25	92	3.01	52.5
$CaCl_2$	12.2	5.72	84	3.36	59	2.87	50
$MgSO_4 \cdot 7H_2O$	19.1	5.72	84	1.75	30.5		
KCl	11.5	5.72	84	5.18	90.5	3.15	55

* 1 cc propylene glycol containing tocopherylhydroquinone was added to 9 cc water or water containing the salts as shown.
† For purposes of brevity, tocopherylhydroquinone is designated as THQ.
‡ Control, water only.

lene glycol-water emulsions. Propylene glycol-water emulsions have been used routinely in this laboratory for the daily intravenous administration of tocopherylhydroquinone in rabbits. One rabbit received daily injections of the emulsion for 7 weeks without signs of irritation to the blood vessels(1). It would appear that such emulsions are useful for parenteral administration in animals. Since the media commonly employed in enzyme studies contain electrolytes, the effect of ions on the propylene glycol-water emulsion was studied. The concentrations used were identical with those employed in Krebs-Ringer Solution. The data given in Table II indicate that the addition of electrolytes results in early separation of the emulsion.

Summary. A relatively stable emulsion of α-tocopherylhydroquinone in propylene glycol and water can be formed. Best results were obtained when propylene glycol solutions containing 100 mg/cc of the hydroquinone were used and the concentration of propylene glycol in the emulsion was 10%. Such emulsions contained 90% of the added hydroquinone and were relatively stable. The emulsion was useful for intravenous administration in rabbits and produced no significant local reaction in the blood vessels. The emulsion has not been administered parenterally in patients; its injection by the intravenous route in man must await further investigation, and

the development of satisfactory methods of sterilization.

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Unusual Antigenic Variation in an Enteric Bacterium. (17944)

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The bacterium to be described is represented by 8 cultures, all of which were isolated by Hinshaw and McNeil(1) in their study of salmonellosis of reptiles. The 8 cultures were isolated from the viscera of as many fence lizards *(Sceloporus occidentalis)* captured along the banks of a stream in Davis, California. According to a personal communication from Dr. Hinshaw, the viscera of each animal were ground, placed in the tetrathionate broth of Kauffmann for enrichment and plated on brilliant green agar. Although the organism was isolated repeatedly from lizards captured at intervals in the same locality, there was no evidence that it produced disease. From the meager information available, this organism would seem to be commonly present in the lizards in that locality.

The bacterium was a motile rod which possessed the usual characteristics of the *Enterobacteriaceae.* It produced hydrogen sulfide, was methyl red positive and Voges-Proskauer negative, failed to form indol or to liquefy gelatin, and gave negative reactions in Stern's glycerol-fuchsin broth. The organism promptly produced acid from d-tartrate and mucate but did not attack l- or i-tartrate. Citrate was utilized as evidenced by growth on Simmons' medium but in fluid medium some of the cultures did not completely utilize the substance. Acid and gas were produced from glucose, arabinose, xylose, rhamnose, trehalose, maltose, sucrose, raffinose, sorbitol, dulcitol and mannitol within 24 hours. Cellobiose was fermented after 5 days. Lactose, inositol, and salicin were not attacked. The cultures possessed a well developed avidity for sucrose, after overnight incubation marked acidity and moderate gas formation were observed in sucrose broth. These characteristics indicate that the organism is an intermediate coliform bacterium which is not readily classifiable in any of the presently recognized genera of the *Enterobacteriaceae.* It was recognized by Hinshaw and McNeil that the cultures were agglutinated by O serum for group B *Salmonella* but no further observations concerning their antigenic characteristics were recorded. Upon examination it was found that the strains were agglutinated to the titre of group B serum and possessed antigens IV, XII_1, XII_2. Mirror absorption tests with serums prepared from *Salmonella abortus-equi* and from the lizard strains established the identity of the O antigens of the two types.

The first cultures received were poorly motile and continuous passage through semi-solid agar was necessary to maintain the cultures in a condition in which they were flocculated well by H serums. The reactions obtained with Pc 231 were typical of those of all the cultures studied and are recorded in Table I. It was found that prompt agglutination occurred in polyvalent *Salmonella* serum and in *Salmonella typhi* serum (d). In addition, the cultures were flocculated slowly and in low dilution in serum for phase 1 of *Salmonella reading* (e,h). Thus it seemed that the cultures possessed antigens d,e,h and that antigen d was the major component while

1. Hinshaw, W. R., and McNeil, E., *J. Bact.,* 1947, v53, 715.