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Stabilization of the Thromboplastic Lipid by Hydroquinone.

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The instability of the thromboplastic lipid has been recognized for many years. McLean[1] demonstrated the progressive loss of thromboplastic activity of cephalin and, by concomitantly measuring its degree of unsaturation, came to the conclusion that there was direct relation between activity of cephalin and its degree of unsaturation. Similarly, Hanzlik and Weidenthal[2] showed that cephalin loses activity in 2 months.

In previous studies on the thromboplastic lipid[3] we were faced with the problem of assaying preparations over long periods of time. An effort was made therefore to protect the active agent from autoxidation using various known antioxidants. Studies were carried out with thiourea, ascorbic acid, acetone bisulfite, and hydroquinone. Of these agents, hydroquinone was by far the most effective. Deutsch *et al.*[4] had previously found that hydroquinone inhibited phospholipid oxidation catalyzed by ascorbic acid. Similarly, Rusch and Kline[5] reported the inhibition of phospholipid oxidation by polyphenolic compounds including hydroquinone. Experiments were accordingly undertaken to find the conditions necessary to prevent the autoxidation of the thromboplastic phospholipid and to determine if the thromboplastic activity would be stabilized under these conditions.

Methods. The lipid preparations used were mixtures of lecithin and cephalin isolated from beef brain and found to have considerable thromboplastic activity.[3] The mixture is a crude one and is designated by the term phospholipin for convenience.

The degree of unsaturation was determined by the Hanus iodobromine method (U. S. Pharmacopea, XI), care being taken to insure at least a 60% excess of the reagent. In the case of preparations protected against autoxidation it was found necessary first to remove the hydroquinone by washing with acetone. In all cases iodine numbers are expressed as grams of iodine reacting with 100 g of the phospholipin freed from hydroquinone.

In order to add different quantities of hydroquinone to phospholipin, a known amount of phospholipin was placed in a glass-stoppered cylinder containing 100 ml of acetone saturated with hydroquinone. Additional hydroquinone was then added to give the proportions desired and the mixture shaken well for 15 minutes. It was then filtered through a Büchner funnel and the residue dried *in vacuo*. This procedure was found to be sufficient to yield a homogenous mixture of hydroquinone and phospholipin.

The preparations were assayed for thromboplastic activity by determining the coagulation time of recalcified dog plasma with and without the addition of the thromboplastic agent. From these values the percent decrease in coagulation time produced by the agent was calculated and used as the criterion for thromboplastic activity. The technics used were the same as those described previously.[3] Two determinations were done in each case,

TABLE I.
Stability of Untreated Phospholipin.

Days	pH	Iodine No.	Activity % decrease in coag. time
0	6.49	78.4	64.2
2	6.21	77.7	64.4
5	6.30	70.4	68.4
7	5.83	59.9	61.2
10	5.30	58.3	65.1
12	5.19	55.2	57.2
15	4.93	56.8	32.4

[1] McLean, J., *Am. J. Physiol.*, 1917, **43**, 586.

[2] Hanzlik, P. J., and Weidenthal, C. M., *J. Pharm. and Exp. Ther.*, 1920, **14**, 157.

[3] Hays, H. W., and Lein, J., *Arch. Biochem.*, 1945, **7**, 69.

[4] Deutsch, H. F., Kline, B. E., and Rusch, H. P., *J. Biol. Chem.*, 1941, **141**, 529.

[5] Rusch, H. P., and Kline, B. E., *Can. Res.*, 1941, **1**, 465.

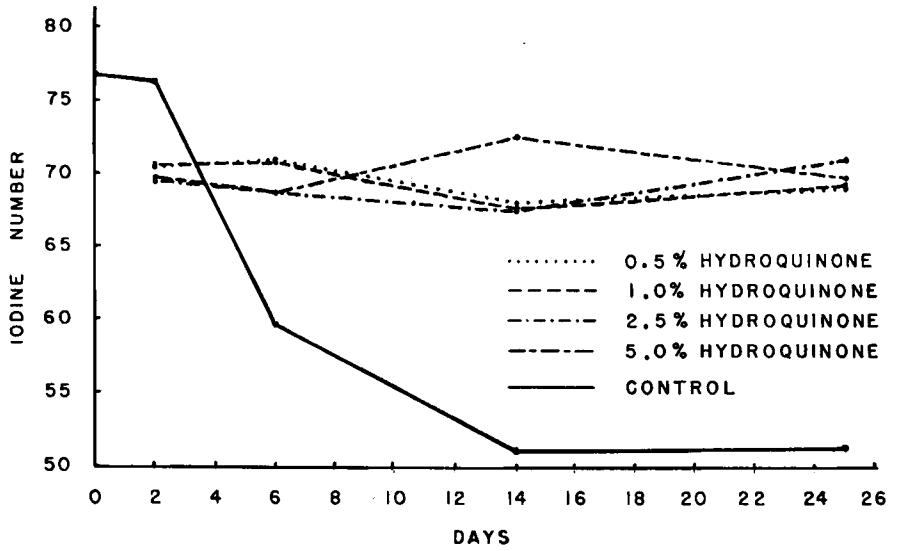

FIG. 1.

The prevention of autoxidation of phospholipin by hydroquinone in concentrations of from 0.5 to 5.0%.

and the average used, providing the deviation did not exceed 10% of the mean. In the few cases where the deviations were greater, additional determinations were carried out.

Autoxidation of Phospholipin. Phospholipin when freshly prepared is granular and has a light cream color. In the course of several days at room temperature and in diffuse light it progressively darkens, becoming orange in color after 7 days, and reddish-brown after 30 days. Concomitant with these color changes, there is a decrease in the pH of saline emulsions of phospholipin, a decrease in its iodine number and a decrease in its thromboplastic activity. Values of these changes in a typical experiment are given in Table I.

The pH values of 0.2% phospholipin emulsions were determined with a glass electrode pH meter. The pH decreased with time, the rate of decrease being greatest at about the tenth day. With a decrease in the pH value, there was a concurrent decrease in iodine number. The iodine number decreased most rapidly between the second and seventh day and tended to reach a stationary level after 12 days. The thromboplastic activity of 0.2% emulsions did not decrease markedly until the twelfth day. This long latent period was in all probability due to the fact that a 0.2% emulsion is in excess of the minimal concentration that would produce maximal activity.[3] Thus, it is evident that phospholipin is very susceptible to autoxidation which markedly alters its physiological as well as chemical characteristics.

Prevention of Autoxidation by Hydroquinone. The amount of hydroquinone added to the phospholipin was expressed as percent of the final hydroquinone-phospholipin mixture. Experiments were carried out using concentrations of hydroquinone varying from 0.5 to 5% and the iodine numbers and thromboplastic activities determined at various time intervals. Parallel determinations were carried out simultaneously on a sample of untreated phospholipin in each series.

Results of the iodine number determinations are presented graphically in Fig. 1. Complete protection against autoxidation by the amounts of hydroquinone used was obtained for 25 days while the untreated control decreased from its original iodine number of 75.7 to a final value of 51.2 in this period of time. It should also be noted that the initial iodine numbers of the hydroquinone-protected phospholipin were considerably lower than that of the control. The reason for this is not

apparent but may be due to small amounts of hydroquinone that resist the washings with acetone prior to the iodine number determinations.

With this stabilization of the iodine number there occurred a stabilization of the thromboplastic activity of the preparations. After the 25 day period the percent decrease in coagulation time produced by the control was only 44 while that of the hydroquinone-protected phospholipin was 80, 79, 79, 80 for the respective 0.5, 1, 2.5 and 5% hydroquinone-phospholipin preparations.

Summary. Hydroquinone in concentrations as low as 0.5% protected a thromboplastically active phospholipid preparation from being autoxidized for a period of at least 25 days. With this protection against autoxidation there was a protection against loss of thromboplastic activity.

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Metabolism of Isocysteine, Labeled with Radiosulfur (S^{35}), in the Rat.

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The administration of isocysteine hydrochloride, α-thiol-β-amino-propionic acid, to rabbits results in a large excretion of "extra" sulfur in the urine.[1] Almost all of this extra sulfur is in organic combination; a large fraction of it is in the form of disulfide which is presumably isocystine, since it does not give the Sullivan[2] reaction. When isocysteine is fed to rats maintained on a diet low in proteins (6% casein), the animals' growth is not increased. The present investigation was undertaken to determine whether the sulfur of isocysteine is oxidized to sulfate by the rat.

Procedure. Isocysteine hydrochloride was prepared from α-bromo-β-alanine and labeled sodium disulfide according to the method of Schöberl and Braun.[3] Five grams of α-bromo-β-alanine hydrobromide and a solution containing 325 mg of disulfide sulfur containing S^{35} equivalent to 130,000 ± 2500 c.p.m. were used in the synthesis. The sulfur in an aliquot of the solution (in 0.1 N sodium hydroxide) of sodium sulfide which was used to prepare the disulfide for the synthesis was oxidized by alkaline fusion. A small amount of carrier was added to the resultant sulfate, and the mixture was precipitated as barium sulfate. This was washed by repeated centrifuging, first with 0.001 N hydrochloric acid and then with water, and transferred as a slurry in 70% alcohol to a tared counting cup, dried, weighed, and counted[4] using a 5/mg/cm² end-window G-M tube and a scaler. Counting was continued for a sufficient time to insure an error of less than 2%. The sulfate later obtained from the specimens of biological material was precipitated, washed and counted in the same way as the sulfate from the original sulfide-sulfur solution except that no carrier was added. All counts were corrected for self-absorption and for decay.

The isocysteine hydrochloride as originally isolated was grossly impure and was purified by conversion to and recovery from its mercaptide. The final yield was 0.88 g (25%) and contained 24% of the initial radioactivity.

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1 Wingo, W. J., and Lewis, H. B., *J. Biol. Chem.*, **1946, 165**, 339.

2 Sullivan, M. X., and Hess, W. C., *Pub. Health Rep.*, U.S.P.H.S. supplement No. 86, 1930.

3 Schöberl, A., and Braun, H., *Ann. Chem.*, 1939, **542**, 274.

4 Dziewiatkowski, D. D., *J. Biol. Chem.*, 1945, **161**, 723.