phyrins to a similar extent as the normal plasma.

The results thus point to the presence of a non-dialyzable inhibitory factor in normal plasma which is possibly a plasma protein or related substance but one apparently not heat-coagulable at a pH 5.5. As can be seen from Table II, however, the possibility of the inhibitory factor being one of the major serum proteins was ruled out by adding electrophoretically pure serum proteins (rat) to the incubation mixture. The serum protein fractions in the concentrations used did not decrease the formation of porphyrins. Anemic plasma did not decrease porphyrin formation under similar conditions. The inhibitory factor, therefore, seems to disappear or to be destroyed or inhibited when the animal is made anemic as in phenylhydrazine-treated dogs.

Since the inhibitory factor is non-dialyzable, ether insoluble, and thermolabile at pH 7.4, it could be some type of plasma protein or a related substance possibly attached to a protein as an association complex. In this connection, Karibian and London(14) have recently presented evidence that hemin itself may have a regulatory effect on porphyrin biosynthesis by a "feedback mechanism." It appears possible that the inhibitory plasma factor observed in the present investigation may indeed be related to the factor described by London and his co-workers, perhaps bound as a protein complex. This inhibitor may play an important physiological role in regulating porphyrin formation to supply normal demands. Thus, when anemic plasma was used, less of the inhibitory factor was present or was less active and more porphyrin formation occurred than when normal plasma was used.

Summary. Evidence is presented which indicates that there is present in the plasma of normal dogs a factor, possibly some type of plasma protein or a related substance, which inhibits *in vitro* biosynthesis of porphyrins from ALA. The amount of activity of this factor is decreased in the plasma of anemic phenylhydrazine-treated dogs. It is suggested that this plasma inhibitory factor may play a physiological role in regulating porphyrinogenesis.

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Serum Vitamin E Determined by Thin-Layer Chromatography. (30588)

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Values in the literature for human serum tocopherol levels have been obtained with analytical methods which yield results expressed as "total tocopherols" (see *e.g.*, 1,2). It would be desirable to know what proportions of the total serum tocopherols are contributed by the various individual tocopherols. This paper describes a rapid, thin-layer chro-

matographic (TLC) procedure which makes possible the separation of α from β, γ and δ-tocopherols.

Methods. For preparation of thin-layer plates, 30 g of silica gel G were placed in a screw-cap bottle and 60 ml of 0.002% aqueous sodium fluorescein added. The contents were shaken vigorously for 1 minute and applied to the plates. Variations in coating thickness did not affect the separation. The 5 × 20 cm plates were lined up lengthwise on the template in order to obtain more uniform coatings. They were dried in an oven at 95-100°C for 2 hours and stored in a desiccator.

One volume of serum (usually 1 or 2 ml) was added to 2 volumes of ethanol containing 10-15 mg pyrogallol in a 15 ml glass-stoppered centrifuge tube. The contents were mixed and the unstoppered tube placed in a 65-70° water bath. After 5 minutes, 0.5 ml of 11 N KOH was added and the stopper was moistened and fixed tightly. The tube was inverted 4 or 5 times to dissolve protein and heating was continued for 25 minutes. After cooling the tube in ice water, 4 ml of hexane and 1 ml of water were added and the tube shaken vigorously for 2 minutes. The tube was centrifuged and a 2 or 3 ml aliquot of the hexane layer transferred to a 5 ml conical centrifuge tube. The solvent was evaporated under a stream of nitrogen in a warm water bath. The lipid residue was concentrated in the tip of the tube by washing the walls down with several portions of hexane. It should be noted that the hexane extract is alkaline and should not be stored for more than 1-2 hours before being analyzed.

The lipid in the 5 ml centrifuge tube was dissolved in 25-30 μl of benzene and applied as a 2 cm streak to one-half of the TLC plate using a 10 μl syringe. Two 10 μl washings of the tube were also applied to the plate. The developing jars, lined with filter paper, contained benzene and were flushed with nitrogen and kept in the dark. When the solvent had moved to within several cm of the top (*ca* 35 minutes), the plates were removed, air-dried for one minute or less and replaced in the same jars for another 35 minutes. After air-drying for 5 minutes the plates were viewed under an ultraviolet lamp (2540 Å); quenching zones were scraped off into tubes and eluted by mixing with 1.5 ml of ethanol. The tubes were centrifuged, 1.0 ml of the ethanol transferred to a 10 × 75 mm cuvette and the Emmerie-Engel (E-E) reaction run as described previously(*2*). Recoveries of α-tocopherol from the TLC procedure averaged 93.7% (range 89.7-95.9).

Results. With standard α, β, γ and δ-tocopherols 3 distinct zones were obtained representing α, $\beta + \gamma$ (not separated) and δ, as shown in Fig. 1. The positions of various unsaponifiable or related compounds are also indicated. Ubiquinone had the same R_f as δ-tocopherol but does not react in the E-E reaction as does ubichromenol. The latter coincided with $\beta + \gamma$-tocopherols and could be a source of error. However, the results from the identification of this band, described below, make it seem unlikely that significant amounts of ubichromenol are present in blood.

In analyses of over 50 sera, when the unsaponifiable fraction from 0.5-1 ml of serum was chromatographed and the plate viewed under ultraviolet light, the only distinct quenching zone in the middle area of the plate was that due to α-tocopherol. In most samples a faint quenching zone corresponding to $\beta + \gamma$-tocopherols was detectable. When sprayed with $FeCl_3$-bipyridyl reagent the α-tocopherol zone quickly became very pink whereas the $\beta + \gamma$-zone only slowly appeared faintly pink or was not apparent. No pink coloration was ever seen in the δ-tocopherol zone.

To verify the identity of the materials on the plate and also to check the quantification by the E-E reaction, the 2 quenching zones and also a third area, corresponding to δ-tocopherol, were obtained from 5 ml of pooled serum and eluted. One-half of each eluate was used for the E-E reaction and the other half was analyzed by gas-liquid chromatography (GLC) using a column of 1% SE-30 as described previously(*3*). The α-zone from the TLC plate gave only one peak (Fig. 2) with the same retention time as α-tocopherol. The $\beta + \gamma$-zone gave a peak corresponding to $\beta + \gamma$-tocopherols

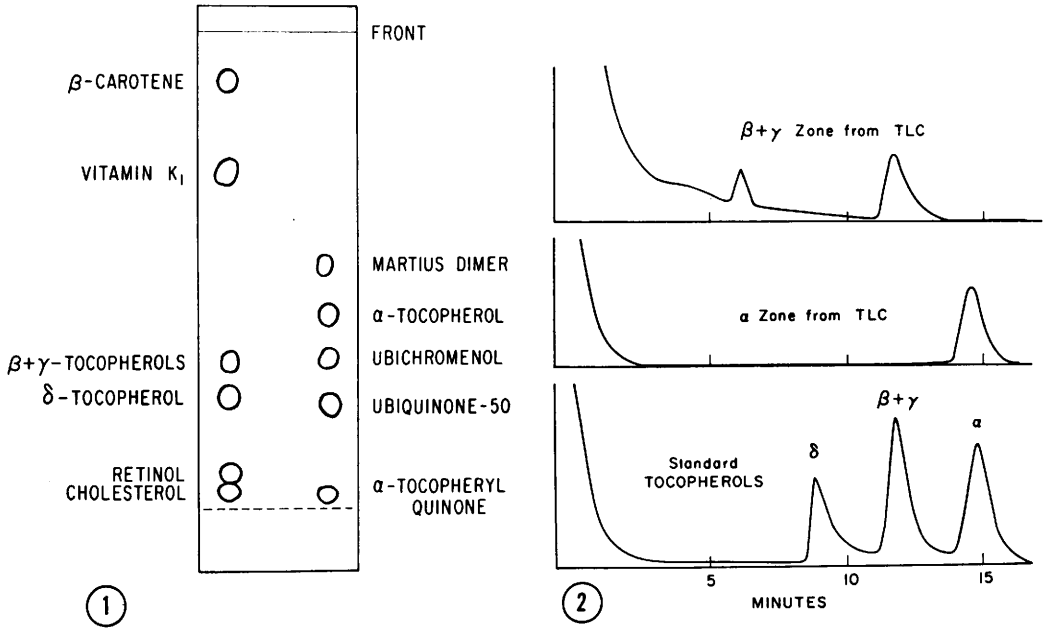

FIG. 1. Relative locations of various lipid soluble compounds on silica gel G, developed twice in benzene.

FIG. 2. Gas chromatographic records of known tocopherols (bottom) and of α- and β + γ-tocopherol fractions obtained from TLC plates.

(these do not separate on the SE-30 column) and also a slower, unidentified peak. This latter appeared in even greater amount in the material from the δ-zone. In several attempts, no evidence for the presence of δ-tocopherol in the slow-moving TLC band was found. Quantification of the GLC α-tocopherol peak gave good agreement with the amounts found in the aliquots by colorimetry (Table I).

A further test of the one-dimensional TLC determination of α-tocopherol was made by comparing it with 2-dimensional TLC. Two ml of serum was saponified and extracted as described and one-half of the extract chromatographed in one dimension and the other half in 2 dimensions using 20 × 20 cm plates. For this latter system, the first solvent was chloroform and the second, isopropyl alcohol-hexane (1:4). The α-tocopherol spots were marked under the ultraviolet light, scraped off, eluted and determined colorimetrically. The results from 6 sera (Table II) gave good agreement between the one- and two-dimensional procedures.

Since the amounts of β- and γ-tocopherols in serum were small compared to α-tocopherol, 5 normal sera were pooled and 10 ml taken for analysis. The unsaponifiable extract was applied to three 5 × 20 cm plates which were developed in one dimension in

TABLE I. Quantification of Serum α-Tocopherol from TLC Plates by Colorimetric or Gas Chromatographic Analysis.*

Serum No.	μg/0.5 ml serum Colorimetric	GLC
1	3.84	4.08
2	3.58	3.85
3	5.61	5.79
4	8.39	8.60

* α-Tocopherol zone from TLC of unsaponifiable fraction was eluted and aliquots determined colorimetrically or by GLC.

TABLE II. Comparison of Serum α-Tocopherol Values Determined by One- and Two-Dimensional Thin-Layer Chromatography.

Serum No.	mg/100 ml serum One dimension	Two dimension
1	370	390
2	650	640
3	700	760
4	1070	1180
5	600	540
6	1460	1300
Mean	808	802

benzene. The β + γ-zone and the δ-zone were eluted separately and reapplied to 20 × 20 plates for 2-dimensional separation. The β + γ sample gave 2 distinct quenching spots with R_f's similar to standard β- and γ-tocopherols. When eluted and determined colorimetrically, the γ-tocopherol corresponded to 56 μg/100 ml serum and the β-tocopherol to 10 μg/100 ml. Thus, the γ-tocopherol comprised about 85% and the β-tocopherol 15% of the reducing material in the combined β + γ zone from one-dimensional TLC.

The δ-zone from the one-dimensional TLC when run in 2 dimensions produced no detectable spot where δ-tocopherol should be, either when viewed under ultraviolet light or when sprayed with $FeCl_3$-bipyridyl. This confirmed the absence of δ-tocopherol as noted above by GLC.

Forty sera from normal, adult employees of the National Institutes of Health were analyzed by the one-dimensional TLC method and also by the "direct" colorimetry procedure, following saponification, as reported in a previous study(2). The results (Table III) with TLC gave for α-tocopherol 0.916 mg/100 ml serum and for β + γ-tocopherols, 0.133 mg/100 ml. Thus, α-tocopherol comprised about 88% of the total tocopherols. The total tocopherols by TLC were 10% lower than by the "direct" procedure. Examination of the α-tocopherol values showed none below 0.5 mg/100 ml and 70% between 0.7-1.2 mg/100 ml. The values for α-tocopherol are somewhat higher than those reported recently by Dayton *et al*(4) and by Herting and Drury(5), both using TLC methods.

TABLE III. Serum Tocopherols Determined by TLC or by Direct Colorimetry (40 Samples).*

Determined by TLC				
α	$\beta+\gamma$	Total	α as % of total	Total direct
.916 ±.304	.133 ±.075	1.049 ±.360	87.6 ±4.4	1.165 ±.351

* Values are means ± S.D., expressed as mg/100 ml serum.

Summary. Forty normal adult sera were analyzed by one-dimensional TLC. The averages in mg/100 ml were: α-tocopherol, 0.916; combined β + γ-tocopherols, 0.133. No δ-tocopherol was found. The procedure was checked by comparing with GLC analyses and also with 2-dimensional TLC.

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Lactic Dehydrogenase Isozymes in Rat Tissues, Tumors, and Precancerous Livers. (30589)

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Lactic dehydrogenase (LDH) activity has been extensively studied in both animal and human tumor bearers. Numerous investigators have reported elevations of enzymic activity in the plasma of animals bearing either primary or transplanted tumors, and recent evidence indicates that this increased activity can be at least partially attributable to a decreased clearance rate of the enzyme from the blood stream(1-4). Starch gel electrophoresis of LDH has revealed at least 5 isozymes, and these are thought to be tetramers of 2 subunits of the enzyme. Synthesis of each of the polypeptide subunits appears to be controlled by a different gene(5-8), and in different tissues each gene shows varying