In conclusion, we believe that: (*a*) the NMR data suggest that theophylline does associate to a limited degree in water but do not permit useful quantitative estimates; and (*b*) for the most practical range of concentrations, the associative tendency of theophylline still appears to be negligible.

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Interference in GLC Determination of Iodoamino Acids in Hydrolysis Products of Thyroid Extracts

Keyphrases ☐ Iodoamino acids—GLC analysis, thyroid extracts ☐ Thyroid extracts—GLC analysis of iodoamino acids ☐ GLC—assay, iodoamino acids

Sir:

The quantitative determination of iodoamino acids in dried thyroid extract is a problem of current interest in many laboratories. The separation and quantitative determination of standard mixtures of the iodoamino acids by GLC were reported by many authors (1–8). However, the extension of these methods to the determination of the hydrolysis products of dried thyroid extract has not been reported.

To shed light on the problems encountered in this determination, we wish to make the following report. In our work, we noticed that our standard mixture of trimethylsilyl derivatives of iodoamino acids did not yield the usual peak areas when injected onto a column that had been used for repeated injections of derivatives of hydrolysis products of dried thyroid extract. However, on injection onto a second column used previously only in the temperature-programming sequence, a return to normal peak areas was obtained (Fig. 1).

Our GLC investigation followed the method of Hansen (7), modified by substituting 1% dimethyl silicone[1] for the stationary phase coated onto a support of acid–base washed, silane-treated diatomaceous earth[2], mesh size 80/100. Three-foot glass U-shaped columns were utilized in this part of the study. A standard derivative mixture was prepared by heating 1.017 mg. of liothyronine (T_3) and 1.510 mg. of thyroxine (T_4) in 100 μl. of anhydrous pyridine and 300 μl. of *N,O*-bis(trimethylsilyl)-acetamide[3] for 2 hr. at 50°.

The first column had some carbon present at the point of injection. To determine if the carbon was interfering with the complete elution of the iodoamino acids, three columns were prepared: one containing the normal

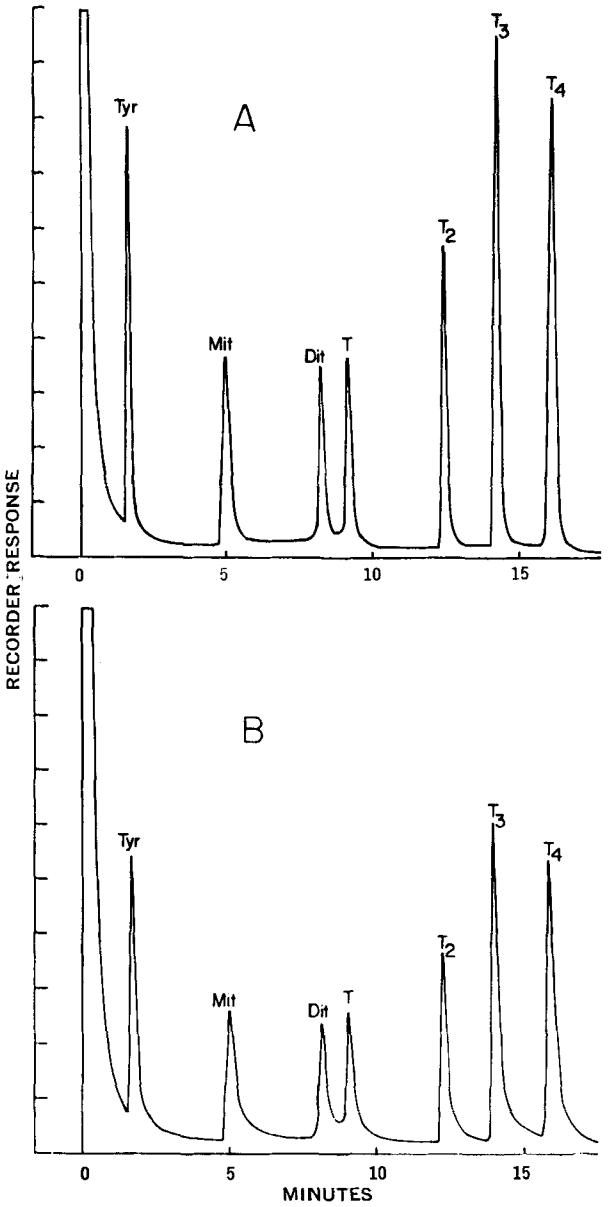

Figure 1—*GLC detection of a synthetic mixture of iodoamino acids on a new column (A) and a used column (B). Key: Tyr, tyrosine; Mit, monoiodotyrosine; Dit, diiodotyrosine; T, thyronine; T_2, diiodothyronine; T_3, liothyronine; and T_4, thyroxine. Chromatographic conditions were: temperature programmed at 165–285°, 3 min. isothermal followed by a programmed rate of 10°/min.; nitrogen carrier gas flow, 100 ml./min. at 165° (40 p.s.i.); injector temperature, 235°; detector temperature, 315°; attenuation, 9 × 10^{-10} amp.; and chart speed, 0.5 in./min.*

[1] OV-1, Applied Science Laboratories, Inc., State College, Pa.

[2] Gas-Chrom Q, Applied Science Laboratories, Inc., State College, Pa.

[3] BSA, Pierce Chemical Co., Rockford, Ill.

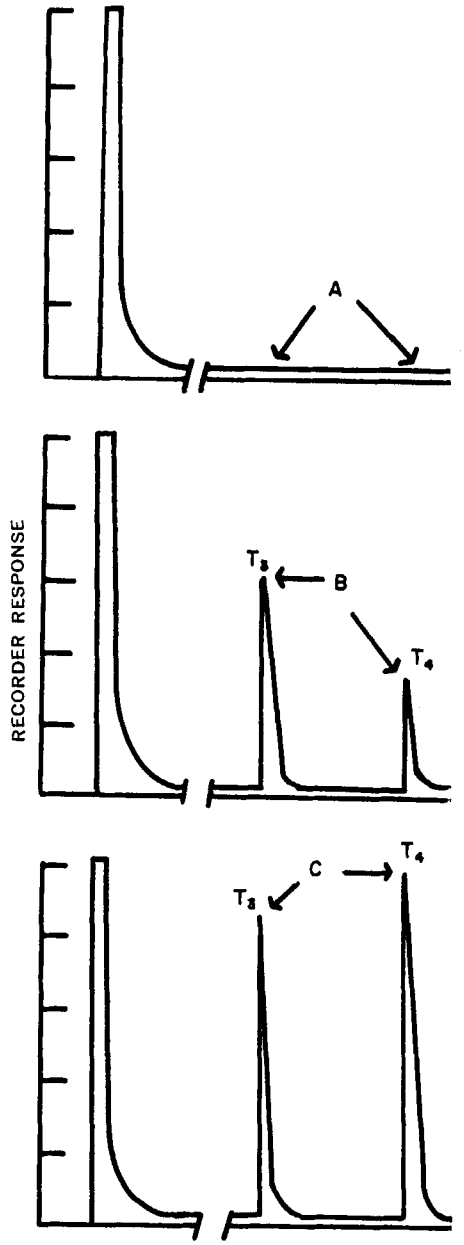

Figure 2—*Composite representation of recovery of a mixture of liothyronine (T_3) and thyroxine (T_4) from GLC columns containing 1.25 mg. (A), 0.125 mg. (B), or no (C) activated charcoal.*

support system previously described, a second with 1.25 mg. of activated charcoal[4] mixed with 50 mg. of this support and placed on top of the column on the injection site, and a third containing 0.125 mg. of activated charcoal mixed with 50 mg. of support and placed on top of the column. All columns were conditioned at 300° for at least 18 hr.

The peak heights obtained on injection of 4 μl. of the derivative mixture into the normal column 1, at an attenuation of 3×10^{-9} amp., were comparable to those expected from previous new columns. No response was observed with the column containing 1.25 mg. of activated charcoal (Fig. 2).

These results appear to substantiate the reasoning that this amount of carbon on the column significantly reduces the ability to detect the iodoamino acids. It was determined that each injection of 3 μl. of hydrolyzed sample deposited at least 1.5 mg. of material on the column. After several such injections, carbon deposits could reach the levels used in the trials. Thus, it may be reasonable to assume that the presence of nonvolatile materials in the protein hydrolysates causes charring on the GLC column and could account for the incomplete recovery of iodoamino acids. This observation may be of some benefit to laboratories involved in this work.

[4] Norit, American Norit, Jacksonville, Fla.

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Assay of Warfarin

Keyphrases ☐ Warfarin—plasma levels, TLC and spectrophotometric assays ☐ Plasma levels—warfarin, assay ☐ TLC—assay, warfarin

Sir:

For the benefit of other investigators interested in the determination of the concentration of warfarin in plasma or stool samples, some comments are in order regarding the work of Welling *et al.* (1). Two of their findings are at variance with our observations.

First, these investigators were unable to detect the presence of metabolites of warfarin in the plasma. We have no experience with the TLC procedure described by Welling *et al.* (1). This procedure utilizes quenching, rather than fluorescence, as the marker for chromatographic loci of interest. In their hands, the same quenching loci were observed in blank plasma and in plasma from subjects receiving warfarin. Our work has been based on a procedure that was outlined in abstract form (2) and subsequently reported in detail (3). After TLC of several hundred extracts of plasma standards (plasma to which warfarin was added), we never observed loci with blue fluorescence with R_f values corresponding to warfarin metabolites. On occasion, with any plasma samples, a clump of fluorescent material runs at the