sodium chloride solution. Most of the livetin remained in solution and so did some of the lipovitellenin (Figure 3, *D* and *E*). Ether extraction changed lipovitellenin from a protein that migrated toward the anode to a nonmobile one (Figure 3, *C*, *D*, and *E*). Vandegaer, Reichmann, and Cook (*16*) observed that the solubility and stability of both lipovitellin and lipovitellenin are impaired by ether extraction.

Prior extraction of egg yolk with ether makes impossible the separation of lipovitellin and lipovitellenin. Only where separations have been made prior to ether extraction have the two been separated. The use of ether by Young and Phinney (*20*) in the preparation of their yolk sample for electrophoresis accounts for their inability to confirm the presence of lipovitellenin in egg yolk.

The data presented indicate that perhaps most of the egg yolk lipides are combined with the proteins and that at least two types of combination exist. The first is a relatively stable combination which is not broken by ether. Lipovitellin and lipovitellenin as commonly obtained are compounds of protein and lipide combined in this way. A weaker lipoprotein-lipide complex also appears to occur, but this complex is broken by contact with ether even in the cold. Recently, Weinman (*18*) as the result of ultracentrifugation studies with unfractionated egg yolk concluded that nearly all egg yolk lipides are bound to protein.

Hawke and Lea (*7*) observed that triglycerides and cholesterol were more readily removed from their lipovitellin preparation by different solvents than were the phospholipides. It has been postulated (*15*) that in lipoproteins the neutral lipide is mainly bound to the phospholipides, and that phospholipides form the link between neutral lipide and protein.

Conclusions

Extraction of egg yolk with ether, either in the cold or at room temperature, changed the electrophoretic behavior of the yolk proteins on paper. Two lipoprotein peaks, lipovitellenin and lipovitellin, were obtained with unextracted yolks, but only one was obtained with ether-extracted yolks. The difference in electrophoretic behavior of ether-extracted yolk proteins and nonextracted proteins was apparently caused by the breaking of an actual lipide-protein bond by the ether. Extraction of lipovitellenin with ether changed its properties so that it gave a peak at the same point as lipovitellin.

Paper electrophoresis methods offer a new procedure for the study of egg yolk proteins. The results of experiments reported herein emphasize the necessity for a new look at the proteins of egg yolk and for the development of isolation techniques that do not involve treatment with ether.

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CORRESPONDENCE

Silicon Determination in Ashed Plant Material

SIR: In the application of the silicon method of Straub and Grabowski [*Ind. Eng. Chem., Anal. Ed.* **16,** 574–5 (1944)] to the analysis of plant material, many ashed samples were found to contain significant amounts of ferrous iron. The presence of this ferrous iron, in conjunction with the phosphorus normally contained in plants, caused falsely high silicon readings. Such results have been reported by Bunting [*Ind. Eng. Chem., Anal. Ed.* **16,** 612–15 (1944)] for samples containing both phosphate and ferrous iron. This difficulty was overcome by the addition of hydrogen peroxide to ensure the oxidation of the iron.

In practice, 50 to 100 mg. of dried and ground tomato plant material were placed in a platinum crucible in a cool furnace and were heated until the temperature just reached 650° C. This heating took about 1 hour. The crucible was immediately removed from the furnace and allowed to cool, after which the ash was washed from the crucible into a polyethylene container. The crucible was then washed with 1 ml. of 2.4*M* hydrochloric acid, and this acid, together with subsequent distilled water rinsings, was added to the ash suspension. One milliliter of 0.15% hydrogen peroxide (30% hydrogen peroxide diluted 1 to 200) solution was added, and the volume was made to 25 ml. From this point, the analysis was carried out as outlined by Straub and Grabowski (starting with the addition of ammonium molybdate), except that half of the recommended volume of each reagent was used, to conform to the 25-ml. sample volume. The strength of the hydrogen peroxide solution was not critical—solutions of 0.03% and of 1% being satisfactory. If solutions much stronger than 1% were used, however, the final reduction of the silicomolybdate was prevented.

The amount of plant material that could be analyzed depended largely upon its phosphorus content, as more than about 50 micromoles of phosphate drastically reduced the final silicon reading. This factor limited the size of sample to 100 mg. in most cases. The lowest reliably detectable amount of silicon by this method was about 5×10^{-9} gram atom in 100 mg. of dried plant material, or about 1.5 p.p.m.

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