Table 6 Group Separation of Amino Acids

System as in Table 5	Separation[a]	Amino acids resolved
A	I	Leu, Phe, Trp, Ala, Glu, Ser, Lys, Cys, Tyr
	II	Leu, Phe, Trp, Thr, Lys
B	I	Leu, Phe, Tyr, Val, Glu, Asp, Lys
	II	Leu, Phe, Val, Trp, Thr, Lys
C	I	Trp, Ile, Val, Ala, Ser, Cys, Lys
	II	Trp, Ile, Val, Thr, Lys
D	I	Trp, Ils, Val, Ser, Glu, Arg, Lys
	II	Trp, Ile, Val, Thr, Lys
FX_A	I	Thr, Gly, Val, Glu, Met, Leu, Phe, His, Lys, Arg
	II	Thr, Val, Met, Leu, Phe, His, Lys, Trp
FX_B	I	Asp, Thr, Gly, Val, Met, Leu, Tyr, His, Lys, Trp
	II	Thr, Val, Met, Leu, Phe, His, Lys, Trp
FX_C	I	Asp, Thr, Gly, Val, Met, Leu, Tyr, His, Lys, Trp
	II	Thr, Val, Met, Leu, Phe, His, Lys, Trp

[a]Group I: 18-component mixture of amino acids. Group II: Mixture of essential amino acids.
Source: Adapted from Ref. 69.

two-dimensional chromatography of the main protein amino acids on Whatman DEAE–cellulose. Kraffczyk and Helger (74) used a double layer consisting of a 2 cm band of cellulose + cation exchanger (45 + 5 g) in aqueous CM cellulose (0.05%), with the remaining portion of the layer prepared from cellulose SF suspension. A mixed layer of cellulose and the ion exchanger Amberlite CG-120 was effectively used in a similar way by Copley and Truter (75). A laboratory experiment was devised for students to illustrate qualitative determination of amino acids in egg lysozyme (75a). Amino acid separation on a newly synthesized support named aminoplast (75b) was compared with that of starch and cellulose using *n*-butanol–acetone–water (4:3:3) and propan-2-ol–formic acid–water (8:1:2). Nevertheless, silica gel continued to be the most widely used and most successful material.

In studies of collagen metabolism, proline and hydroxyproline were separated by TLC on silica-impregnated glass fiber sheets with 2-propanol–water (7:3), located by spraying with ethanolic ninhydrin reagent and autoradiography, and recovered by dialysis (75c). Amino acid mixtures were analyzed by separation on C_{18} layers with MeOH–water (1:1, 1:3, 1:5) mobile phases, detection with ninhydrin, and derivative spectrometry of the colored reaction products (75d).

III. SEPARATION OF AMINO ACID DERIVATIVES

Separation and identification of derivatives of amino acids such as DNP, PTH, dansyl, and DABITC amino acids is very important, particularly in the primary structure determination of peptides and proteins. Adequate descriptions of the preparation of PTH (76–79), dansyl (80–82), and DNP amino acids (83–86) are available in the literature, and the methods of identification of N-terminal amino acids by TLC and other techniques have been reviewed by various workers (87–91). The present section describes briefly the preparation of such derivatives and TLC resolution data reported in recent years.

When an $—NH_2$ group of an amino acid at the N-terminal end of a polypeptide (or a free molecule) is coupled with phenyl isothiocyanate, the corresponding PTH derivative is obtained. The sequential degradation of amino acids as their PTH derivatives from a polypeptide followed by their identification is used to establish the primary structure of proteins (76). Both manual and automated and liquid-phase and solid-phase methods are currently used for small and large poly-