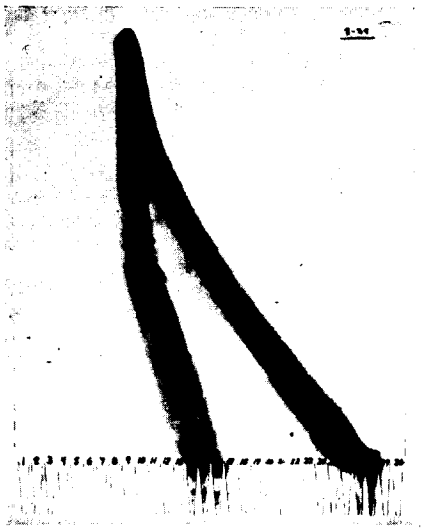

Fig. 1. Continuous electrophoretic separation of two strains of TMV. Strain U-1 migrated to tubes 26–28; strain U-2 migrated to tubes 13–17.

The solutions which collected in tubes 13–18 were pooled, as were the solutions which collected in tubes 25–29. The two solutions were dialyzed against *M*/90, pH 6.9, phosphate buffer for at least 72 hours to remove a nitrogen-containing substance of unknown composition which comes from the paper. After centrifugation at 2500 r.p.m. for 20 minutes in a clinical centrifuge, the amount of protein-nitrogen in each supernatant solution was determined. Tubes 13–18 contained a total of 10.5 mg of virus-protein, and tubes 25–29 contained 3.4 mg. Although equal amounts of each strain were applied to the paper it was commonly observed that strain U-2 moved to the bottom of the paper more rapidly than strain U-1; thus relatively more of strain U-2 collected in the tubes. Consequently, less U-2 remained on the paper, which accounts for the lighter staining of the U-2 band in Fig. 1.

In other experiments it was shown that the total yield of virus could be greatly increased if the process of elution of the virus from the paper was allowed to continue for several days.

An aliquot of each pooled solution (before dialysis) was tested to determine whether a biological separation of the two strains had been achieved. A few ml of each pooled solution was mixed with a small amount of celite and each was rubbed onto the leaves of several *Nicotiana rustica* (var. Olsen 68) plants. After 7 days the symptoms which appeared on the plants indicated that the two isolates were biologically distinct. The *N. rustica* plants which were rubbed with the solution from tubes 13–18 developed symptoms which were typical of those obtained with pure strain U-2; *i.e.*, small necrotic lesions developed on the inoculated leaves with no subsequent systemic infection. The plants which were rubbed with the isolate from tubes 25–29 showed markedly different symptoms, typical of those obtained with purified strain U-1 when it is inoculated into *N. rustica*. Local lesions appeared at the point of inoculation, but these spread rapidly and a systemic infection developed which, after 21 days, had killed the apex of the plant.

In the light of current experiments it is envisaged that the technique of continuous filter-paper electrophoresis can have wide application in the separation of mixtures of plant viruses and of virus strains, and in the separation of plant viruses from their accompanying host-proteins.

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N-terminal residues of serum lipoproteins

The shifts in lipoprotein pattern observed during the clearing reaction have led to the suggestion that low-density lipoproteins are interconvertible *in vivo*[1]. It has also been reported that α-lipoprotein concentrations may rise at the expense of low-density lipoprotein concentrations during *in vitro* clearing[2–3]. If interconversion occurs directly, through stripping down of lower density material by delipidation, the protein moieties of source and product should be identical. The amino acid composition of several lipoprotein fractions has been reported to be almost identical[4]

but this in itself cannot, of course, constitute proof of identity. Conversely, even though the molecular weights of the protein moieties are apparently different this cannot be taken to rule out interconversion because of the possibility that the proteins are made up of sub-units. The present studies, demonstrating distinct N-terminal residues in the β_1- and α_1-lipoproteins, show structural difference of their protein moieties and thus rule out direct interconversion, at least for these two materials.

Pooled Red Cross serum was ultracentrifugally fractionated to obtain lipoproteins with density 1.019–1.063 (β_1-lipoprotein) and 1.063–1.21 (α_1-lipoprotein). These fractions correspond closely to Gofman's Sf 0–12 and "high-density lipoproteins" respectively[5]. Each material was recentrifuged for purification and the concentrated solutions were dialyzed against physiological saline. Dinitrophenylation was carried out at pH 9 and 40° C according to the method of LEVY[6]. In some cases this was done on the intact lipoprotein and in others after partial extraction of the lipid moiety with cold ether. The results were not materially altered by prior delipidation. The DNP-protein was hydrolyzed 16 hours at 105° C in 6 *N* HCl and the DNP-amino acids were identified and quantitated[6]. The system of BLACKBURN AND LOWTHER[7] was used for final identification of DNP-aspartic and DNP-glutamic acid. Correction factors for losses during hydrolysis were those of PORTER[8].

As shown in Table I, glutamic acid appears to be N-terminal in the β_1-lipoprotein (D 1.019–1.063) while aspartic acid is N-terminal in the α_1-lipoprotein (D 1.063–1.21). Essentially similar results were obtained on lipoprotein fractions isolated from the serum of a single donor.

TABLE I

DNP-amino acids recovered	*moles/mole protein**	
	D 1.019–1.063	*D 1.063–1.21*
Glutamic acid	0.49	trace
Aspartic acid	trace	0.76
Serine	0.05	0.04
Threonine	0.05	0.05
Leucine	trace	0.0

The molecular weight of the protein moiety in the D 1.019–1.063 fraction (300,000) is taken from the data of ONCLEY *et al.*[9,10]; that of the D 1.063–1.21 fraction (100,000) is based on the molecular weight of the whole lipoprotein determined ultracentrifugally[9] and a direct measurement of the protein content of the samples analysed (50%).

It has been well established that the clearing reaction is enzymically catalyzed and that the primary reaction is the hydrolysis of triglycerides to yield free fatty acids and glycerol[11]. The removal of triglycerides from high molecular weight, low-density lipoproteins by such a reaction accompanied by concomitant changes in the make-up of the other lipid fractions could lead to an increase in higher density lipoproteins. The structural difference between the β_1-lipoprotein protein and the α_1-lipoprotein protein demonstrated here rules out such a mechanism, at least with regard to these two classes. The demonstration by KORN that β_1-lipoprotein is a poor substrate for the clearing factor enzyme also rules against such a possibility. Interconversion by a more complex mechanism in the whole animal must still be considered. Tracer experiments in this laboratory have shown no significant *in vivo* interchange of labeled protein moieties between α_1-lipoprotein and β_1-lipoprotein in rabbits[12].

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