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Lipoproteins in Atherosclerosis: a Comparison of the Results of Paper-electrophoresis with those of Ultracentrifugal Analysis in a high-density Medium

By **A. Fasoli, F. Salteri,** and **A. Cesana**

Attempts to fill the need for a practical and reliable "screening test" to reveal the presence of lipoprotein abnormalities in patients with clinical evidence of atherosclerosis, have been made with several different methods: their results, though not in contrast, do not strictly correspond.

Ultracentrifugal flotation, zone-electrophoresis, and chemical fractionation, have been employed extensively; each of these methods can be performed with different technical procedures. For instance, media of different density may be used for the ultracentrifugal study; the various fractions can be determined with optical or chemical methods. Zone-electrophoresis has so many variants, that it would take a long time only to list them: however, electrophoresis on filter-paper, followed by staining with a dye of the Sudan series, and by densitometric scanning, is being used so extensively, because of its simplicity, that a comparison of the results so obtained with those of a method more reliable from a quantitative point of view, such as ultracentrifugal analysis, might be useful.

We shall present data obtained in a series of 60 atherosclerotic patients and 22 normal individuals, in whom the lipoprotein pattern was determined both with paper-electrophoresis (Sudan IV staining: *Fasoli* and *Salteri* [1]), and with ultracentrifugal analysis in a KBr-NaCl medium (Density = 1.21: method of *Green*, *Lewis* and *Page* [2]): the notation $-S_{1.21}$ indicates the flotation rate in this medium, expressed in Svedberg units.

The fractions of the electrophoretic lipoprotein pattern were divided into three groups: A–α_1 (fractions migrating with albumin and α_1-globulin, plus the fast-moving "F"-fraction, when present), α_2–β_1, and

β_2 (non-migrating fraction). The ultracentrifugal fractions were divided into three classes, too: $-S_{1.21}$ 0–15 (high density lipoproteins), $-S_{1.21}$ 15–35 (corresponding to the fraction S_f 0–8 of *Gofman*) and $-S_{1.21}$ 35–400 (corresponding to the S_f 8–100 of *Gofman*); within this last class, the $-S_{1.21}$ 70–400 fraction has been distinguished, for particular purposes.

A direct comparison of the results of the two methods is presented in figures 1 and 2, where the A-α_1 electrophoretic fraction is plotted against high-density lipoproteins ($-S_{1.21}$ 0–15) (Fig. 1), and the β_2 fraction is plotted against the $-S_{1.21}$ 70–400 (Fig. 2). This coupling was selected because high-density lipoproteins are known to have the electrophoretic mobility of α_1 globulin, while the β_2 electrophoretic fraction, being rich in neutral fat, should contain the lowest density components, such as the ones of the $-S_{1.21}$ 70–400 class.

Fig. 1 demonstrates that a fairly good correlation exists between high-density lipoproteins and the A–α_1 electrophoretic fraction.

On the contrary, a very poor correlation, if any, is shown by fig. 2 to exist between the $-S_{1.21}$ 70–400 class and β_2 lipoproteins. This finding is

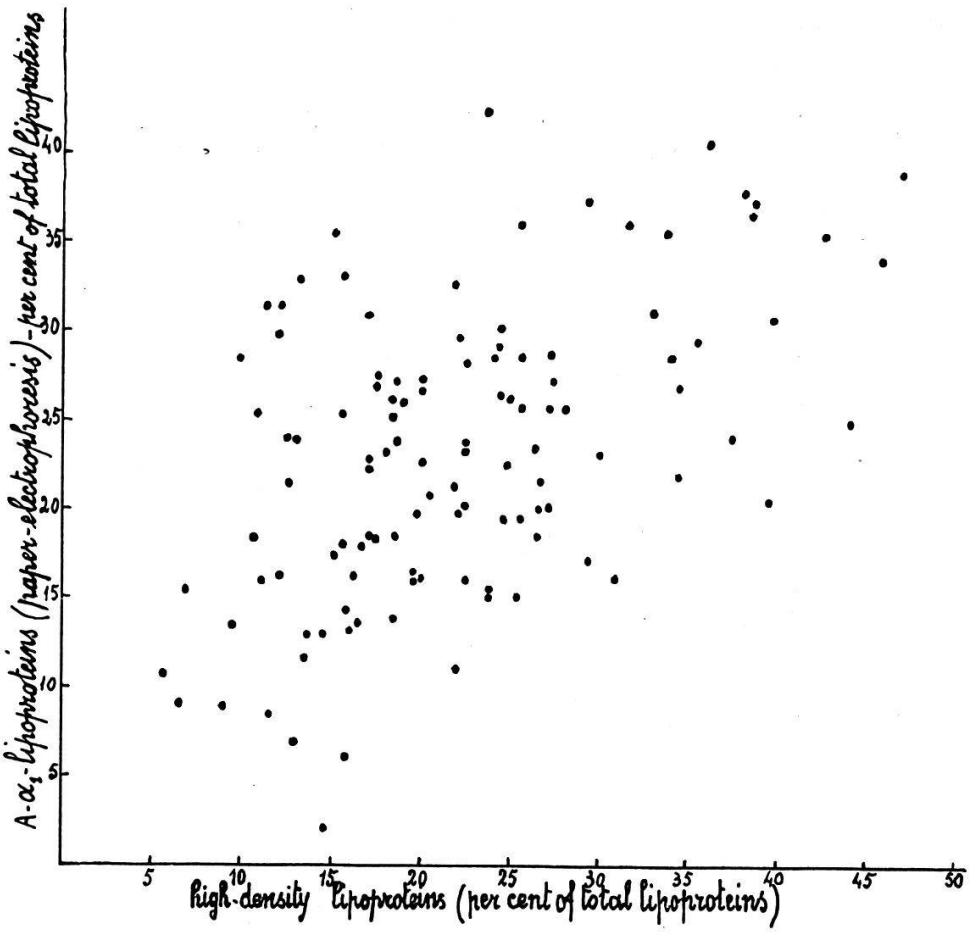

Fig. 1. Correlation between high-density lipoproteins (ultracentrifugal analysis) and the A-α_1 fraction (electrophoresis on filter-paper; Sudan IV staining), in 127 normal and pathological sera.

not unexpected, since the adsorption phenomenon that gives origin to the β_2 component is not strictly specific for a particular class of lipoproteins, though large molecules are involved more than smaller ones; furthermore, uncontrolled factors may interfere with the extent of adsorption.

Figs. 3, 4, and 5 show the results of both methods in several groups of normal and pathological sera.

In fig. 3 are reported the levels of high-density lipoproteins and of the A-α_1-fraction in normal men and in patients with clinical signs of atherosclerosis or with hypertensive heart disease. In general, the average values of high-density lipoproteins for the various groups closely correspond to the average levels of the electrophoretic A-α_1 fraction for the same groups. Statistical analysis (method of the "t" of *Student*), shows that the differences between each group of patients and the normal series are significant with both methods; the highest degree of significance concerns the youngest age group of atherosclerotics.

Fig. 4 represents the values of the $-S_{1.21}$ 35–400 class and of the β_2-fraction in the same cases of fig. 3. Here, ultracentrifugal and electrophoretic data show a different behaviour. In fact, the $-S_{1.21}$ 35–400 class values are significantly increased in all the groups of atherosclerotic patients, more markedly in the youngest age groups; on the contrary, the β_2-fraction, while, on the average, higher in atherosclerotic than in normal individuals, does not show consistent and significant changes:

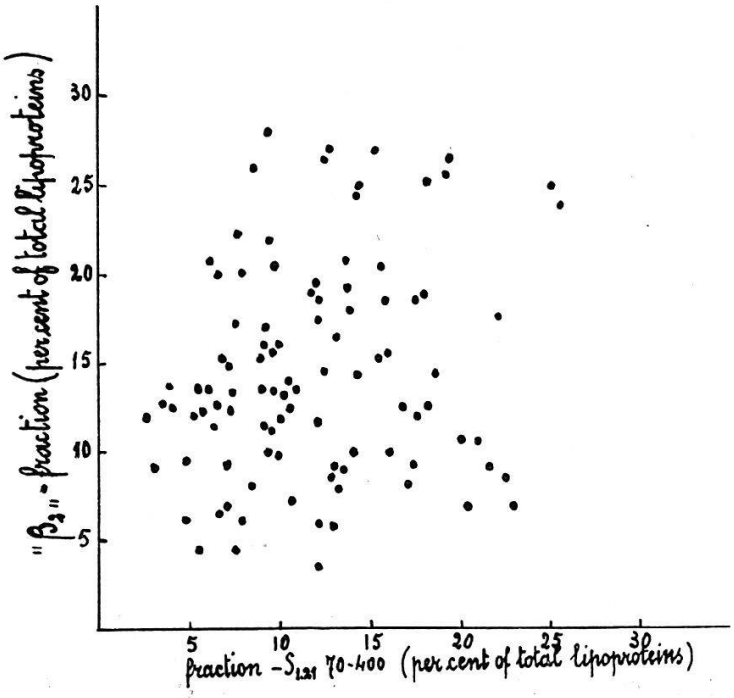

Fig. 2. Correlation between the $-S_{1.21}$ 70–400 ultracentrifugal fraction and β_2 lipoproteins in 103 normal and pathological sera.

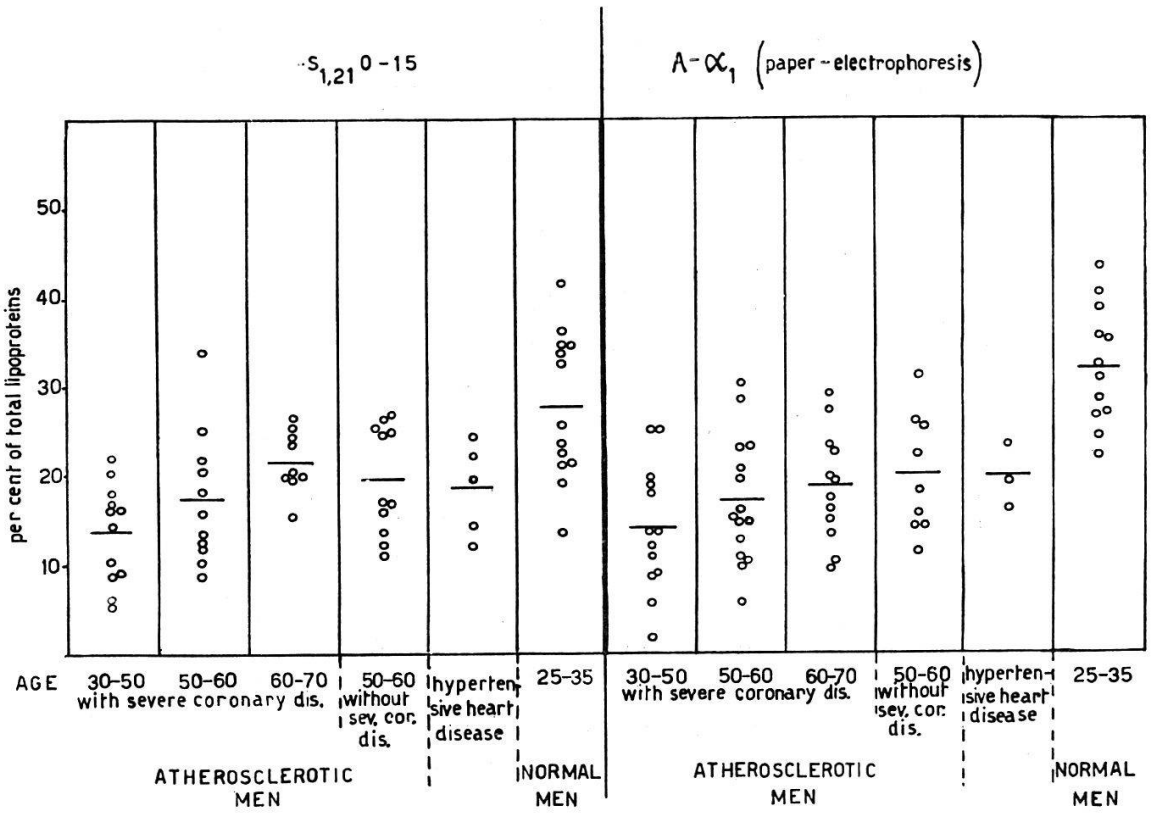

Fig. 3. High-density ($-S_{1.21}$ 0–15) and A-α_1 lipoprotein levels in normal and atherosclerotic men.

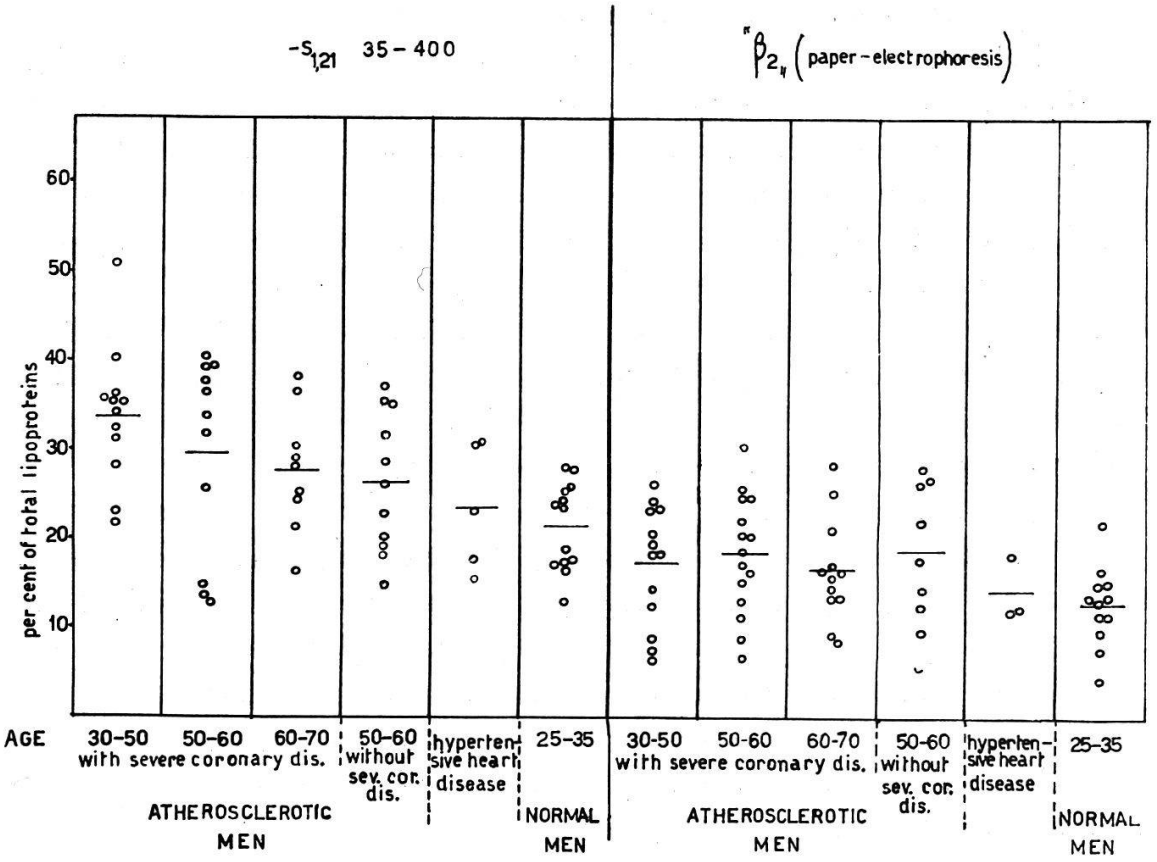

Fig. 4. The levels of $-S_{1.21}$ 35–400 and β_2 lipoproteins in normal and atherosclerotic men.

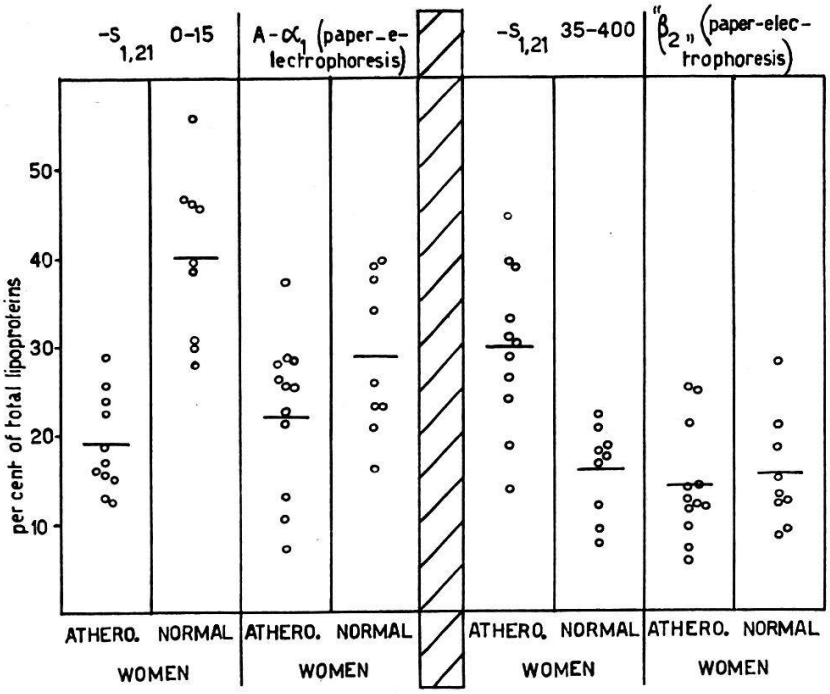

Fig. 5. The levels of $-S_{1.21}$ 0–15 and 35–400, of A-α_1, and of β_2 lipoproteins in normal and atherosclerotic women.

only for the group of atherosclerotics with coronary disease of the 50–60 years age group, a significant P value was obtained (P = 0.02).

Total serum cholesterol determinations, performed simultaneously, showed changes of the average values in the same direction as the $-S_{1.21}$ 35–400 class, but of smaller degree and less significant statistically.

Fig. 4 shows the levels of the $-S_{1.21}$ 0–15 and 35–400 classes and of the A-α_1 and β_2 fractions in normal and atherosclerotic women. In this case, too, high-density lipoprotein changes correspond to similar changes of the A-α_1 fraction; the normal values are higher, in this series, for high-density lipoproteins than for the A-α_1 fraction, without an apparent reason. A marked and highly significant rise, above the normal, of the 35–400 class is observed in atherosclerotic women, while no significant difference from the normal results from the study of the β_2 fraction.

Our results can be summarized as follows:

1. Consistent changes of high and low density lipoproteins are present in patients with clinical evidence of atherosclerosis. It is difficult to establish, at present, whether low or high density lipoprotein changes are directly connected to atherogenesis. However, the decrease of the high density class, being observed in many unrelated conditions, seems to be less specific: it is probably to be considered as an index of the severity of the metabolic derangement.

Patients of the youngest age group show marked changes both of high and low density lipoproteins, while in patients of the 60–70 years age

group, the low density lipoprotein changes prevail over those of the high density group, that are often indeed absent: this different behaviour might suggest that, while in young patients a severe derangement of lipid metabolism is usually present, probably atherosclerosis is associated, in the aged patient, only with a slowing down of the disposal of neutral fat containing lipoproteins.

2. The results of paper-electophoresis are parallel to those of ultracentrifugation as far as α_1 lipoproteins are concerned. Subfractionation of β lipoproteins on the basis of adsorbability on filter-paper does not afford results consistent with ultracentrifugal flotation data, nor are the results useful "per se", in segregating atherosclerotic from normal individuals.

The only reliable information that can be obtained from paper electrophoresis, with regard to atherosclerosis, seems, therefore, to be expressed by the β/α ratio, as previously suggested by other authors (3, 4). On the other hand, the fact that the diminution of A-α_1 lipoproteins, although it indicates a serious derangement of the mechanism of lipid transport, is not specific of atherosclerosis or known atherogenic conditions, suggests that changes of the β/α ratio are to be considered only as an indirect criterion of the presence of possibly atherogenic alterations of plasma lipoproteins.

Summary

For the investigation of plasma lipo-proteins, different methods were used depending on different principles: fractionation with organic solvents, zone-electrophoresis, ultra-centrifugation in high density media. The results obtained with these techniques agree in general, but as yet there are no investigations with direct comparison of the results of different methods, particularly with regard to the evaluation of the lipo-protein changes which can be shown in atherosclerotic cases by zone-electrophoresis and fractionation in ethanol (increase of the β/α ratio) and with ultra-centrifugation after Gofman (increase of the S_f 10–100 fractions) on the other side.

The object of the investigations reported here was to compare the lipo-protein spectrum obtained with paper electrophoresis, through the usual methods of lipid staining, with that obtained by analytic ultracentrifugation, the latter being carried out in a solvent of density 1.21 (according to *Green*, *Lewis*, and *Page*) to determine the fractions of low density (β-lipo-proteins) as well as those of high density (α-lipo-proteins). The results obtained from 200 simultaneous determinations on the serum of 60 atherosclerotic patients and 22 healthy subjects, showed a

remarkable agreement of the two methods in demonstrating a decrease of the content of α_1-lipo-proteins in the atherosclerotic cases which was the more marked the earlier the atheromatous process appeared (group of under 50 years of age).

An analogous agreement was found between the increase of lipoproteins with $-S_{1.21} > 35$ (S_f 10–100 of Gofman) and the early occurrence of the atheromatous processes. The non-migrating fraction of paper electrophoresis (β_2 or «trail») usually shows variations which agree with those of the fractions $-S_{1.21} > 35$, but only in direction and not in amount. The results are discussed in detail and also in connection with the significance of the lipo-protein changes in atherosclerotic patients and with regard to the value of the different methods for these investigations.

Zusammenfassung

Für die Erforschung der Plasmalipoproteine sind verschiedene, auf verschiedenen Grundsätzen beruhende Methoden angewandt worden: Fraktionierung mit organischen Lösungsmitteln, Zonenelektrophorese, Ultrazentrifugierung in Lösungsmitteln hoher Dichte. Die mit diesen Techniken erhaltenen Resultate stimmen im großen und ganzen überein. Bisher aber fehlen Untersuchungen mit direkten Gegenüberstellungen der Ergebnisse der verschiedenen Methoden, speziell in bezug auf eine Bewertung der betreffenden Lipoproteidveränderungen, die bei Atherosklerotikern mit Zonenelektrophorese und der Fraktionierung in Etanol (Zunahme des β/α Quotienten) einerseits und mit der Ultrazentrifugierung nach Gofman (Zunahme der S_f^0 10–100 Fraktionen) anderseits nachgewiesen werden können.

Aufgabe der hier referierten Untersuchungen war der Vergleich des mit Papierelektrophorese nachgewiesenen Lipoproteidspektrums, bei welchem für die Färbung der Lipoide die gewöhnlichen Methoden angewendet wurden, mit den durch analytische Ultrazentrifugierung gewonnenen Ergebnissen. Die Ultrazentrifugenuntersuchungen wurden in einem Lösungsmittel von der Dichte 1,21 (nach *Green, Lewis* und *Page*) ausgeführt, um sowohl die Fraktionen niedriger Dichte (β-Lipoproteide) als auch jene von hoher Dichte (α-Lipoproteide) zu bestimmen. Die Resultate, die bei 200 gleichzeitigen Bestimmungen aus Seren von 60 Atherosklerotikern und 22 Gesunden gewonnen wurden, zeigen eine bemerkenswerte Übereinstimmung der beiden Methoden beim Nachweis der Abnahme des Gehaltes an α-Lipoproteiden bei Atherosklerotikern, die um so ausgesprochener ist, je früher der atheromatöse Prozeß beim Kranken aufgetreten ist (Gruppe der unter 50jährigen). Wir haben

außerdem eine Zunahme der Lipoproteide mit $-S_{1.21} > 35$ (S_f 10–100 nach *Gofman*) bei frühzeitigem Auftreten des atheromatösen Prozesses beobachtet. Die bei der Papierelektrophorese nicht wandernde Fraktion (β_2 oder «Trail») stellt meistens mit den Fraktionen $-S_{1.21} > 35$ übereinstimmende Variationen dar; es handelt sich aber nur um eine Übereinstimmung in der Richtung, aber nicht um eine Übereinstimmung in der Größe der Variationen, welche im allgemeinen bei der elektrophoretischen Komponente mengenmäßig gering ist.

Die Ergebnisse werden in den Einzelheiten besprochen, auch in bezug auf die Bedeutung der lipoproteinämischen Veränderungen bei Atherosklerösen und in bezug auf den Wert der verschiedenen Methoden für diese Untersuchungen.

Résumé

Pour l'étude des lipoprotéines plasmatiques, diverses méthodes ont été employées, basées sur des principes variés: le fractionnement par des solvants organiques, l'électrophorèse zonale et l'ultracentrifugation en milieu à haute densité. Les résultats obtenus par ces techniques concordent dans les lignes générales, mais, jusqu'à présent, la confrontation directe de ces diverses méthodes n'a pas été étudiée en ce qui concerne particulièrement les altérations des lipoprotéines mises en évidence chez les artérioscléreux par l'électrophorèse zonale et le fractionnement dans l'éthanol (augmentation du rapport β/α) et l'ultracentrifugation d'après Gofman (augmentation des fractions à S_f 10–100).

Le but de nos recherches était de comparer le spectre lipoprotidique mis en évidence par électrophorèse sur papier, en employant les méthodes de coloration des lipides avec celui obtenu par ultracentrifugation analytique en solvant de densité 1,21 (selon *Green*, *Lewis* et *Page*), afin de déterminer, soit les fractions de faible densité (lipoprotéines β), soit les fractions de forte densité (lipoprotéines α). Les résultats obtenus dans 200 analyses simultanées sur les sérums de 60 sujets artérioscléreux et de 22 sujets normaux ont montré une concordance remarquable des deux méthodes dans l'évidentiation d'une diminution du taux des lipoprotéines α_1 chez les artérioscléreux, diminution d'autant plus marquée que le début du processus athéromateux intervenait à un âge plus jeune (groupe d'âge inférieur à 50 ans). Une concordance analogue a pu être démontrée entre l'augmentation des lipoprotéines à $-S_{1.21} > 35$ (S_f 10–100 d'après Gofman) et la présence du processus athéromateux chez les sujets plus jeunes. La fraction non migrante dans l'électrophorèse sur papier (β_2 ou «trail») présente la plupart du temps des variations, qui concordent qualitativement avec celles de la fraction

$-S_{1.21} > 35$. Suit une discussion détaillée des résultats et de la signification des altérations lipoprotéinémiques dans la pathogenèse de l'artériosclérose et de la valeur des diverses méthodes employées pour leur étude.

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