PLASMA PROTEIN VARIABILITY IN MONOZYGOTIC TWINS: FURTHER STUDIES AND APPLICATIONS

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Previous collaborative studies have shown the substantial superimposability of electrophoretic patterns of non-Ig plasma proteins in twin pairs only if MZ. *The above findings provide the basis for* (a) *twin pair zygosity determinations and* (b) *studies aimed at discriminating genetic and environmental factors in the alteration of protein patterns in various diseases (especially neoplastic). Such studies require adequate standardization of methods, and the present paper offers a substantial contribution to the standardization of pattern comparisons.*

INTRODUCTION

A previous paper presented at the First International Congress on Twin Studies (Milani-Comparetti and Saccucci 1974) stated the general principles underlying our study in the following terms: " According to the central dogma of molecular biology, the genome controls metabolism through protein synthesis. Thus in a mixture of metabolites every individual exhibits a prevalence of genetic conditioning, up to the point of permitting biological identification if the components of the mixture are in sufficient numbers...". The same paper presented evidence indicating that superimposability of most segments of a polyacrylamide gel column after electrophoresis of plasma proteins was to be expected only if the twins were MZ. No attempt was made at the time to define the methodology for interpair comparisons.

In the present paper we report on a series of comparative analyses, on a limited sample of twin pairs, intended mainly as a contribution towards standardization of what we believe to be a potentially useful research tool.

MATERIAL AND METHODS

A sample of 25 twin pairs, belonging to two age groups, 40-70 and 5-12 years old, has been considered. Zygosity determination was based on the analysis of genetic markers and other criteria, as defined by Gedda et al. (1974).

The method for polyacrylamide gel electrophoresis is the one adopted by Sega et al. (1971); a detailed description in English is available upon request from our laboratory.

Our assumption being that superimposability would occur mostly in intrapair comparisons of MZ pairs (probably excluding the immunoglobulin-containing slow fractions), we proceeded to compare the distribution of stained bands in the gel by three separate methods: (*a*) direct, visual comparison of the gel

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columns; (*b*) comparison of densitometric patterns obtained on an automatic integrating densitometer (Cellomatic, Ciampolini, Florence); and (*c*) comparison of the distribution of relative fronts (Rf) as evidenced in the densitometric patterns.

RESULTS

Figs. 1-3 show, respectively: (1) the triplicate gels for a pair of MZ twins; (2) the superimposed patterns of triplicate gels; (3) the superimposed patterns of the two twins. Note the similarities between twins but slight inequalities between repetitions. Figs. 4-5 represent the gels and patterns for a DZ pair (note the obvious differences). Tables 1-3 represent the comparisons in the distribution of Rf values.

Our hope was that densitometric patterns would make comparisons easier than direct visual observation of the gels, and Rf comparisons would be even better. As things stand now, the results appear quite the opposite. Starting from Rf distributions, it is quite true that some MZ pairs exhibit nearly complete coincidence of bands (Table 1), but others (Table 2) exhibit " shifting " of entire segments in opposite directions: here segmental series of bands do exhibit coherent behaviour, but the " shifting " phenomenon interferes with quantitation of band concordances. We have found no menas, as yet, to distinguish the differences due to " shifting " in the similar patterns of MZ pairs from those due to the dissimilar patterns for DZ pairs. We are currently experimenting the application to our problem of the methods of numerical taxonomy: the problems are practically the same and the solutions ought to be the same.

Table 1. *Comparison of Rf values in one MZ twin pair (B. Giuliana and Giorgia)*

	Rf1	Rf2	Rf3	Rf4	Rf5	Rf6	Rf7	Rf8	Rf9	Rf10	Rf11	Rf12	Rf13	Rf14	Rf15	A
GIU.	6	7	14	20	24	27	32	37	41	46	60	64	77	82	84	100
GIO.	5	9	11	22	24	26	33	39	43	47	60	64	77	81	84	100

Table 2. *Comparison of Rf values in another MZ twin pair (P. Giovanni and Giacomo) with band " shifting "*

	Rf1	Rf2	Rf3	Rf4	Rf5	Rf6	Rf7	Rf8	Rf9	Rf10	Rf11	A	PreA
GIO.	5	11	15	20	28	37	46	54	59	67	78	100	115
GIA.	6	11	23	38	45	55	60	68	80			100	115

Table 3. *Comparison of Rf values in a Dz twin pair (D.A. Angiola and Rosetta)*

	Rf1	Rf2	Rf3	Rf4	Rf5	Rf6	Rf7	Rf8	Rf9	Rf10	Rf11	Rf12	A	PreA
A.	5	7	13	20	28	37	45	55	60	71	76	80	100	107
R.	5	8	17	20	22	26	30	40	45	58	61	80	100	103

Intrapair comparisons between gel columns and between densitometric patterns are reported in Table 4.

Gel columns and patterns were divided in five segments (A = albumin; pA = post-albumins; T = transferrin; pT = post-transferrins; L = last, or slow, fractions). Comparisons for each segment were scored as follows: 1 = similar; 2 = different; 0 = non comparable.

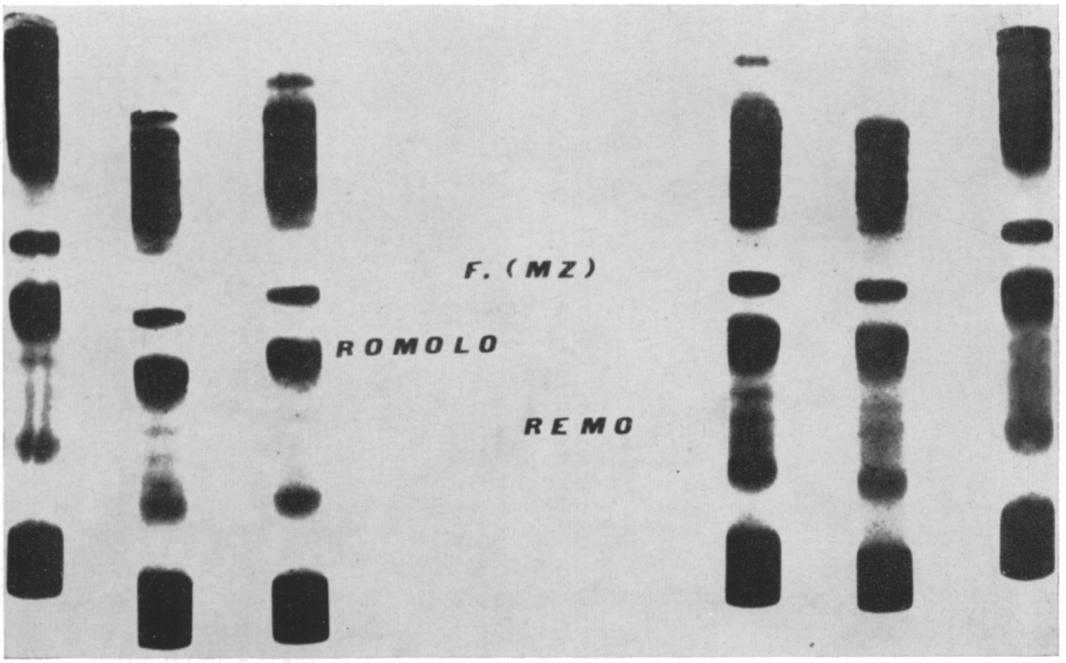

Fig. 1. Triplicate gel columns for one pair of MZ twins.

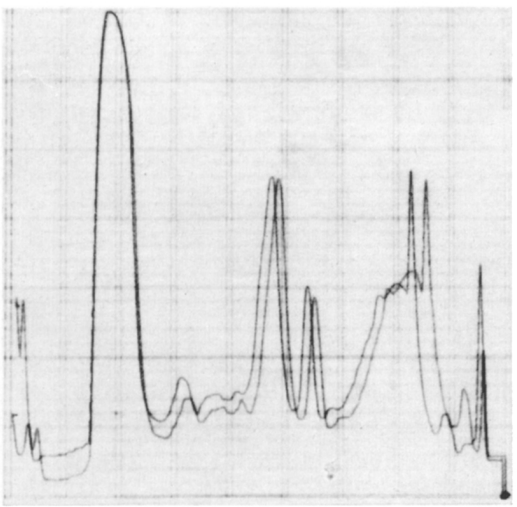

Fig. 2. Superimposed densitometric patterns for one twin in Fig. 1.

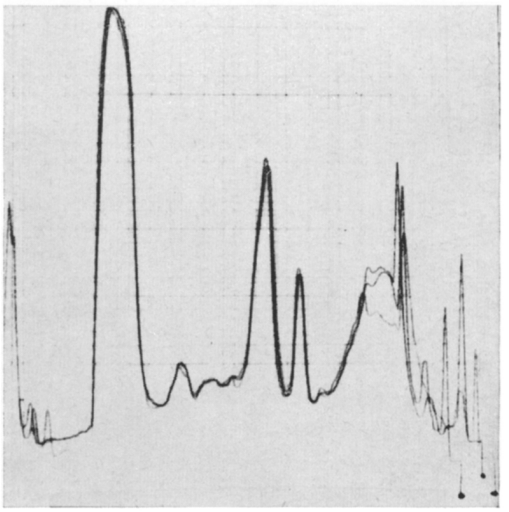

Fig. 3. Superimposed densitometric patterns for two MZ twins.

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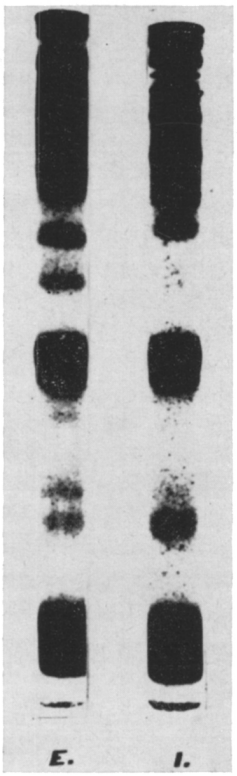

Fig. 4. Comparison of the columns for one pair of DZ twins.

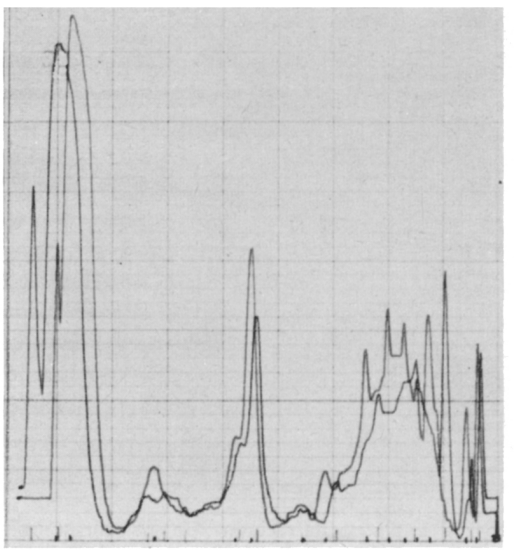

Fig. 5. Superimposed densitometric patterns for two DZ twins.

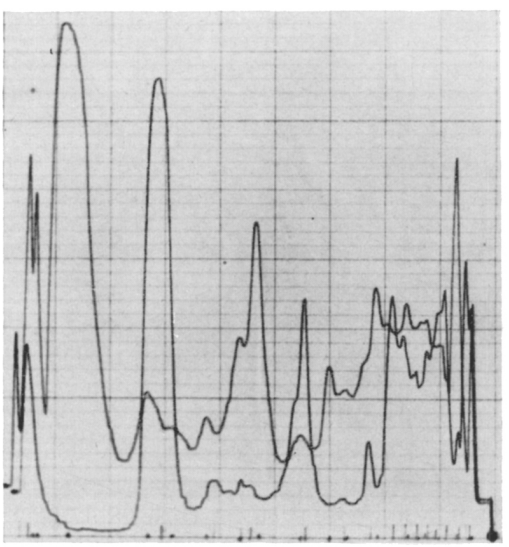

Fig. 6. Superimposed densitometric patterns for the same sample: one fresh and one following long freezer preservation.

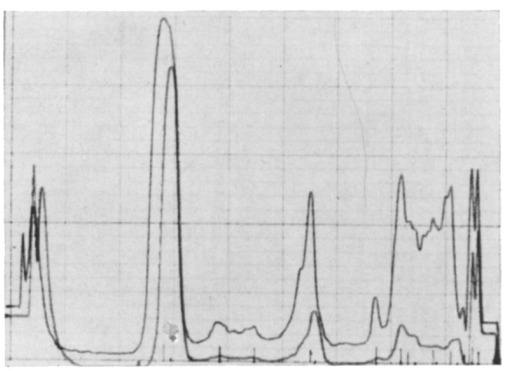

Fig. 7. Superimposed patterns for two MZ twins: fresh samples.

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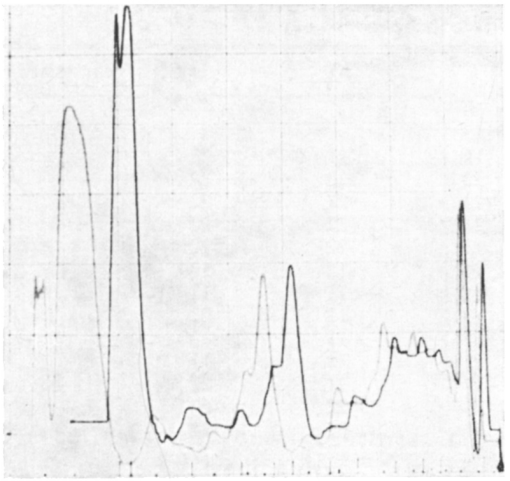

Fig. 8. Superimposed patterns for the same pair as in Fig. 7, after long freezer preservation.

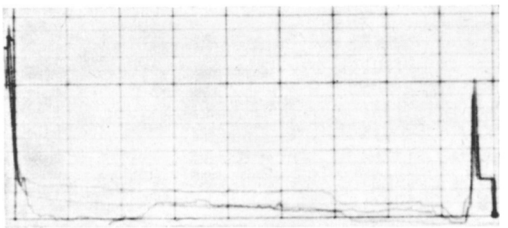

Fig. 9. " Background noise " in blank gel densitometric patterns.

In each column of the Table left digits refer to the densitometric pattern and right digitis refer to the gel columns.

For the younger sample, double comparisons are listed: one for fresh samples (1974) and one for the same samples after two years in the freezer (—30° C). Some methodological problems were in fact involved: would it be feasible to compare samples drawn at different times? And would the gels remain unaltered for a long time?

Figs. 6-8 give some indications in this respect: in Fig. 6 we see the same sample giving quite different results when electrophoresed with a two-year interval; the same finding applies to our other sera; Fig. 7 indicates substantial MZ pattern superimposability in the " fresh run " sample, as previously published; Fig. 8 indicates that superimposability in the same pair does remain when tests are made with serum kept for two years in the freezer.

These findings, to be verified in cooperation with our colleagues in the department of biochemistry, suggest that alterations do occur in freezer conservation (polymer break-down?), but these alterations are relatively constant, thus making comparisons possible between preserved sera, as long as time and conditions are the same, while comparisons between fresh and preserved sera should be avoided.

The subjective comparisons reported in Table 4 were tested for operator bias: no significantly contrasting appraisal was recorded by three operators adopting the same criteria.

More discordances were observed in the densitometric patterns than in the gels. After weeding out sources of error, such as traffic vibrations and false peaks due to handling damage, scanning of fully treated but blank gels (Fig. 9) does in fact reveal " ground-level " irregularities that are totally absent in visual comparisons. A possible solution to this problem lies in reflectance scanning of column photographs: this would increase complication and costs but might reduce another drawback of densitometric patterns, i.e., excessive base spreading of darker bands, an optical phenomenon tending to minimize or mask adjacent lighter bands. As things stand now, visual observation of the columns still appears to provide the best comparisons.

Table 4. *Distribution of subjective appraisal of intrapair concordance/discordance in different segments of the gel columns*

Twin pair no.	A	pA	T	pT	L	Year tested
A. Twins Aged 40-70						
12050	11	11	11	11	22	
10376	21	22	22	22	22	
2063	22	20	22	22	22	
12202	11	22	22	22	22	
13288	11	11	11	22	22	
177	11	11	11	12	12	
10217	11	11	11	11	12	
11101	22	12	12	12	22	
505	11	11	11	11	12	
1551	11	11	11	11	11	
115	11	11	11	21	22	
12714	12	11	21	22	22	
13673	22	22	22	22	22	
10941	11	12	11	12	22	
B. Twins Aged 5-12						
8629	21	22	22	22	22	76
8629	11	11	21	11	22	74
12314	21	22	12	22	22	76
12314	11	11	12	00	00	74
10413	21	11	21	11	22	76
10413	21	11	11	11	11	74
10951	22	20	22	22	22	76
10951	21	22	21	22	22	74
8666	21	12	10	10	20	76
8666	11	11	11	11	11	74
12734	22	12	11	12	12	76
12734	21	11	11	11	21	74
9081	21	21	11	11	11	76
9081	11	11	11	11	11	74
10464	21	11	11	11	22	76
10464	21	11	11	11	22	74
8692	11	11	11	12	22	76
8692	22	11	11	11	11	74
12901	11	11	11	12	12	76
12901	11	11	11	11	20	74

Left figure: comparison of densitometric pattern; right figure: comparison of the gel column.
A: albumin; pA = post-albumins; T: transferrin; pT = post-transferrins; L = last (slow) fractions.

We are now experimenting with thin layer electrophoresis and electrofocusing with promising results, as reported in a forthcoming paper, but costs and complications again seem to be higher with those techniques for many laboratories. Much as we realize the difficulties in optimizing our present method, we do believe it has good potential for research and we shall try to improve on it.

The comparisons listed in Table 4 were analyzed in different ways, and here we report on one method: we recorded the "pooled" comparisons for each segment, comparing them with the expected concordances on the basis of zygosity. The results are listed in Table 5, indicating that:

(1) The A and L segments are hardly correlated with zygosity, and should be excluded from intrapair comparisons.

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Table 5. *Ratio of experimental/expected concordances, for different gel segments, in the hypothesis of DZ = 2 and MZ = 1 in Table 4*

Segments	Twins aged 5-12		Twins aged 40-70
	1974	1976	
A	2/8	1/8	8/12
pA	6/8	5/8	10/12
T	6/8	6/8	9/12
pT	6/8	6/8	6/12
pA+T	6/8	8/8	10/12
pA+pT	6/8	6/8	8/12
T+pT	6/8	8/8	8/12
pA+T+pT	6/8	7/8	9/12
L	5/8	3/8	4/12

(2) The best correspondence between protein pattern and zygosity is found in the long central segment extending from pA to pT. A possible source of error in our appraisal may come from inclusion in the pT segment of part of the immunoglobulins.

(3) The best correspondence is found in the 1976 columns for the younger twins. This may reflect improvements in the method and confirms the validity of long-frozen sera.

(4) The lack of full correspondence in the adult twin sample might be due to defects in the "pooling" procedure, but it may also reflect gradual loss of protein pattern concordance. This is being verified as related to the health status of the twin pairs involved, according to our initial hypothesis.

(5) Whenever all central segments concur in either concordance or discordance, this agrees with zygosity diagnosis, confirming the validity of the method for this purpose.

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RIASSUNTO

Variabilità delle Proteine Protoplasmatiche in Gemelli Monozigotici

Precedenti studi, hanno dimostrato la sostanziale sovrapponibilità del tracciato elettroforetico delle proteine plasmatiche non immunoglobuliniche nelle coppie gemellari *solo se MZ*. Su tale premessa si basa sia la determinazione dello zigotismo nelle coppie gemellari e sia un piano di ricerca per discriminare le componenti genetiche ed ambientali nelle alterazioni del quadro proteico in malattie diverse, soprattutto neoplastiche. Per tali ricerche è fondamentale la standardizzazione della metodologia, ed il lavoro rappresenta appunto un concreto contributo alla standardizzazione del confronto fra tracciati.

RÉSUMÉ

Variabilité des Protéines Plasmatiques chez les Jumeaux Monozygotiques

Des études préalables, ont confirmé que les tracés electrophorétiques des protéines non-Ig du plasma humain sont presque superposables chez les jumeaux seulement s'ils sont monovulaires. Ces résultats ont permis d'envisager soit la détermination du zygotisme des jumeaux et soit des études visant à discriminer les facteurs de l'hérédité et de l'environnement dans les altérations du profile protéique dans plusieurs maladies (surtout néoplastiques). Ces études exigent une standardisation des méthodes, et ce rapport vise à contribuer à la standardisation de la comparaison entre tracés.

ZUSAMMENFASSUNG

Die Variabilität der Protoplasmaproteine bei EZ

Frühere Untersuchungen haben gezeigt, daß sich die elektrophoretischen Kurven der im Blutplasma enthaltenen Proteine (mit Ausnahme der Immunoglobuline) bei Zwillingen nur dann decken, wenn es sich um EZ handelt. Auf diese Voraussetzung stützt sich sowohl die Eiigkeitsbestimmung der Zwillingspaare als auch ein Forschungsprojekt zur Entdeckung der Erb- und Umweltskomponenten, die bei verschiedenen Krankheiten, vor allem bei Neoplasien die Veränderung des Eiweißbildes bewirken. Für derartige Forschungen ist es äußerst wichtig, über eine Standardmethodik zu verfügen und die vorliegende Arbeit trägt in konkretem Maße dazu bei, den Vergleich von Kurven in der Methodik zu standardisieren.

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