Protein profiles derived by automated immune precipitation

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This presentation will cover much the same ground as Professor Laurell's but from a slightly different viewpoint. There is no great difference between us, though we use agarose gel electrophoresis only to detect paraproteins. Like others, we feel that scanning the electrophoretogram contributes nothing to the interpretation of the test¹, as even with high resolution systems the various fractions are composed of mixtures. Instead, we prefer to estimate specific proteins by the automated immunoprecipitation system, and we routinely estimate 12 plasma proteins (albumin, α_1 -antitrypsin, haptoglobin, orosomucoid, α_2 -macroglobulin, transferrin, complement C3, complement C4, IgG, IgA, IgM, LDL protein) and can estimate more than 30. The same proteins could of course be estimated by the techniques outlined by Dr Laurell. Specific protein analysis is superior to electrophoresis in that in addition to giving information on individual proteins it is quantitative. It is also reliable over a wide range of concentrations, it will differentiate proteins which occupy the same electrophoretic zone, and it is applicable to samples which may be unsatisfactory for electrophoresis due to haemolysis, etc. It cannot, however, replace electrophoresis in the identification of small paraproteins, particularly of free light chains which cannot be detected with the present AIP system, nor in the study of genetic polymorphism when the total amount of protein in different specimens may be the same even though they contain different molecular variants.

Before discussing the interpretation of the profiles obtained by specific estimation of 12 proteins using current methods, it is pertinent to consider the quality of the analytical results obtained. About 18 months ago, the Centre for Disease Control in Atlanta, Georgia, distributed to many laboratories in the United States three samples of serum containing known amounts of IgG, IgA, and IgM for analysis. The great majority of the laboratories used

radial immunodiffusion, and a few used the AIP system. The results for IgG (fig 1) were poor, to say the least; whereas the actual value was about 11.5 g/l, the observed values ranged from 7.5 to 18 g/l. Results from the five reference laboratories were somewhat better than this. The results for IgA were similar; for one sample containing about 1.45 g/l IgM, some laboratories found less than 0.75 g/l and others more than 3.0 g/l, although the reference laboratories were all close to the true figure. For another specimen with an IgM content of about 45 g/l a large number of laboratories found the IgM content to be zero and only a few laboratories obtained values within an acceptable range (fig 1). Had the operator merely looked at the sample, it would have been obvious that there was something seriously abnormal because the sample had the consistency of syrup. Electrophoresis would have shown that almost all of the plasma protein was IgM.

There are probably several causes of these poor results, including faulty technique, but in this particular circumstance the companies which manufacture the plates containing antibody must take a share of the blame.

Interpretation of the results of protein estimation is almost always based on a comparison of values expressed in g/l or units, with a 'normal range' determined by measurements on a heterogeneous population. However, a number of factors will influence the levels found in such a population and must be taken into account when results are interpreted. Most of us would accept that age, ethnic group and sex all influence the serum levels of the immunoglobulins. Other factors include upper respiratory and gastrointestinal viral infection, pregnancy and oral contraceptives.

When considering individual proteins the question arises whether to correct for the total protein concentration which may be falsely lowered, eg, by parenteral administration of fluids, or falsely elevated, eg, by venous stasis during sampling or evaporation in the laboratory. The problem remains whether the observed value for total protein

¹The importance of scanning for the estimation of paraproteins is emphasized by Dr Carter and Dr Kohn.

Fig 1 Results obtained for IgG and IgM in sera distributed to many laboratories. Ig3-001, Ig3-002, and Ig3-003 represent three different sera.

should be used or the value obtained after subtracting the amount of any paraprotein from the total.

Ideally, interpretation should be based on the normal values for the particular individual, but such information is rarely available, in contrast to clinical data such as the patient's normal height and weight,

etc. Well-person laboratory screening will be necessary to provide such base-line values.

The limitations of a normal range determined in a population is illustrated by the results of serum protein estimation in 2200 randomly selected people (fig 2). The distribution is very wide for some pro-

Fig 2 Distribution of serum protein concentrations in 2200 randomly selected people.

teins, eg, IgA. An even wider range could result if measurements happened to be made during a period when there were many 'normals' with sub-clinical upper respiratory or gastrointestinal infections. Some proteins, such as α_2 -macroglobulin, have a bimodal distribution corresponding with subjects over or under 21 years of age, but the ranges in the two age groups overlap. Thus, a population norm is only of limited value for interpreting data from an individual, but at the moment it is all that is available.

A further difficulty in determining the normal range is that of deciding whether all values obtained

from an apparently normal population should be included, whether all values from an apparently abnormal population should be excluded, and whether the criterion of 'normality' should be extended to the relatives of the individuals studied. For example, the range of values obtained for serum low-density lipoproteins is very wide, but when values from subjects who have relatives under 55 years of age with cardiovascular disease are excluded, the range is much narrower, although there are still some outliers. Our data on low-density lipoproteins show a distinct difference between the prepubertal individual, the middle-aged and the aged. We have a fairly large population aged over 80 in northern New England and we found that the low-density lipoprotein levels for those aged over 70 approximated to those of adolescents, the levels in middle age being somewhat higher. This raised the possibility that those with the higher values in middle age had less chance of reaching the 70s, and posed the question, Should they be treated prophylactically? We found outliers, even in children, supporting the notion that the latter may have biochemical disease for two or three decades before the first signs of coronary or cardiovascular disease become manifest; if so, low-density lipoprotein estimations in childhood may enable protective measures to be taken.

It is well known that the concentrations of many proteins alter dramatically in early life, so that in order to interpret any given value properly it is necessary to know the age of the individual. The changes in IgG and IgM are shown in figures 3 and

FIG. 3

Fig 3 Variation of serum IgG concentration (ordinate) with age (abscissa).

Fig 4 Variation of serum IgM (ordinate) concentration with age (abscissa).

FIG. 4

4. In addition to large changes with age, which make interpretation of IgG levels particularly difficult, there is also a very wide range of values at any given age. In order to evaluate the benefit that would be gained by following an individual's values from year to year, we studied individuals at three monthly intervals for many years. The variation was very small in the absence of intercurrent illness; for example, for IgG we found variations of 1.0 to 1.5 g/l as compared with the overall range of 8.0 g/l for a large population. The fact that the physiological variation in an individual is so much less than the range of values found in a population seriously compromises our ability to interpret a single set of values.

We have attempted to assist the physician to overcome some of the difficulties of interpreting laboratory data by displaying them in a manner which is easy to read, and which does not require knowledge of the numerical values or of the normal range. This takes the form of a computer printout, with a bar representing the protein level as a percentage of the normal mean (for the population) running across a colour-shaded background (fig 5); the central zone denotes approximately the normal mean ± 1 standard deviation, the intermediate zones ± 1 to 2 standard deviations, and the outer zones any deviation exceeding ± 2 standard deviations. The mean and standard deviations are adjusted by the computer for various factors where appropriate, such as age and sex. In the case of haptoglobin the distribution of values in a large population is very skewed, so that the normal range cannot be indicated by means of the standard deviation. Our population mean for haptoglobin is

about 1.05 g/l, and 0.2 g/l is still within the normal range. However, if the latter value were displayed in terms of the mean and standard deviation, it would appear to be an abnormally low result. Therefore, in order to display the result in the standard manner, we have to adjust the value for the skewed distribution by means of an equation which brings the result into the low-normal range (fig 5).

Interpretation of the results by computer is an elaborate process (fig 6). The interpretative programme involving only 10 of the 12 proteins amounts to about 150 000 words and requires about 15 seconds to process. The computer may print out up to two pages of information, which is of course too much for anyone to read. The machine goes through about 800 individual decisions, some of which are illustrated in figure 7. In the example shown the first question is, 'Is the IgG value less than or greater than 40% of the "normal", taking account of age etc?' If it is less than 40% the computer would print out 'marked hypoproteinoglobulinaemia involving IgG'. If it is greater than 40% but less than 65% a statement 'significant hypoproteinoglobulinaemia involving IgG' would be printed. These values for 40 and 65% are, of course, only relevant to our particular population.

The importance of correcting for total proteins is illustrated by the profile in figure 7. At first sight it looks abnormal and was reported as such. However, we would now correct this for the raised total protein, which would reduce all the values into the normal zone. The new printout would now say that this study is normal, but would also state that there is probably haemoconcentration. When to correct for alterations in total protein concentration is very

Fig 5 Illustration of the computer printout of serum protein values. The central zone indicates the mean ± 1 SD for a normal population, the intermediate grey zone ± 1 SD to ± 2 SD. The lower display illustrates the effect of a correction by the computer for the skewed distribution of the normal range for haptoglobin.

Age:	adult	Sex:	MALE	Patient No:	314.11	Date:	SEP. 24 1974
Protein		mg %	Low	Normal	High		
ALBUMIN		51.2	*****	*****			
ALBUMIN (EXPSIV)		21.0	*****	*****			
HAPTOGLOBIN		43	*****	*****			
GROSS GLOBULIN (A1AGP)		5.5	*****	*****			
22-MAGNIGLOBULIN		17.2	*****	*****			
TRANSFERRIN		44.8	*****	*****			
COMPLEMENT C3		16.8	*****	*****			
COMPLEMENT C4		5.1	*****	*****			
IMMUNOGLOBULIN G		7.0	*****	*****			
IMMUNOGLOBULIN A		111	*****	*****			
IMMUNOGLOBULIN M		51	*****	*****			
LGL PROTEIN		127	*****	*****			
(BLANK) (51)							
TOTAL IMMUNASSAY (GMS)		7.3	*****	*****			

- THIS STUDY IS ABNORMAL FOR A PERSON OF THIS AGE AND SEX.

OF NOTE:

-NORMAL LEVELS OF IMMUNOGLOBULINS.

-MARKED ELEVATION OF THE TRANSFERRIN VALUE SUGGESTS
DEPLETION OF ACCESSIBLE IRON STORES OR INADEQUATE IRON INTAKE.

-HYPERCHLOMICRURIA WITH NORMAL LOW-DENSITY LIPOPROTEIN.

THE PATIENT HAD NOT YET BEEN FASTED PRIOR TO BLEEDING.

Fig 8 *Example of computer printout*¹.

¹The author has been unable to provide better photographs of the printouts illustrated in figs 7-10 but readers should be able to see the pattern in each.

Age	ADULT	Sex	MALE	Patient No.	566,31	Date	SEPT. 3 1974
Protein	mg %	Low	Normal	High			
ALBUMIN	42.0	*****					
AL-ANTITRYPSIN	23.0	*****					
HAPToglobulin	3.0	*****					
OROSOMUCOID (A1AGP)	61	*****					
A2-MACROGLOBULIN	25.0	*****					
TRANSFERRIN	32.0	*****					
COMPLEMENT C3	133	*****					
COMPLEMENT C4	25	*****					
IMMUNOGLOBULIN G	56.0	*****					
IMMUNOGLOBULIN A	1.0	*****					
IMMUNOGLOBULIN M	17.0	*****					
LUL PROTEIN	1.0	*****					
(PLANAR 42)							
TOTAL IMMUNOASSAY (G%)	7.4	*****					

THIS STUDY IS ABNORMAL FOR A PERSON OF THIS AGE AND SEX.

OF NOTE:

ELEVATED TOTAL PROTEIN AT LEAST PARTIALLY EXPLAINED BY THE FOLLOWING:

THERE IS SUFFICIENT DISPARITY BETWEEN THE LEVELS OF HAPToglobulin AND ONE OR MORE ACUTE PHASE REACTANTS TO SUGGEST THAT THERE IS INTRAVASCULAR RELEASE OF HEMOGLOBIN. POSSIBLE CAUSES ARE:

- 1. THROMBOCYTOSIS
- 2. MECHANICAL AND DAMAGE
- 3. REDUCTION OF A HEMATOMA
- 4. INTRAVASCULAR COAGULATION SYNDROME
- 5. COLD AGGLUTININ HEMOLYSIS

A LARGE "M" COMPONENT IS DEMONSTRATED ON SERUM PROTEIN ELECTROPHORESIS. THESE VALUES ARE CONSISTANT WITH WALDENSTROM'S MACROGLOBULINEMIA.

THE HYPERGAMMA GLOBULINEMIA SHOULD BE SERIOUSLY CONSIDERED. IN ADDITION THERE IS SIGNIFICANT HYPERIMMUNOGLOBULINEMIA INVOLVING IgG.

MAJOR DIFFICULTIES WITH HIGH SYSTEMIC RESISTANCE TO INFECTION ARE NOT ANTICIPATED.

DETAILED URINARY IMMUNOGLOBULIN STUDIES MAY BE OF ASSISTANCE.

Fig 9 Example of computer printout¹.

difficult to assess and to program for but seems to be very valuable.

The situation illustrated in fig 8 is somewhat different. Elevation of transferrin is the only abnormality, and this is an unusual finding in a male. The commonest two conditions in which we have seen this are chronic blood loss and lymphoma. The presence of hyperchylomicronaemia is also noted, probably indicating that the patient was not fasting; this was deduced from the blank value in the system which gives a fairly good estimate of serum triglyceride levels.

Sometimes the report has to be more detailed. An example is given in fig 9 which shows α_1 -antitrypsin falling in the normal zone, haptoglobin falling somewhere below it, and orosomuroid virtually normal. The computer has stated that there is a disparity between the level of haptoglobin and one or more of the other acute phase reactants.

Age	45 YEARS	Sex	FEMALE	Patient No.	524,23	Date	OCT. 8 1974
Protein	mg %	Low	Normal	High			
ALBUMIN	28.0	*****					
AL-ANTITRYPSIN	4.0	*****					
HAPToglobulin	3.0	*****					
OROSOMUCOID (A1AGP)	23.0	*****					
A2-MACROGLOBULIN	32.0	*****					
TRANSFERRIN	12.0	*****					
COMPLEMENT C3	22.0	*****					
COMPLEMENT C4	42	*****					
IMMUNOGLOBULIN G	132.0	*****					
IMMUNOGLOBULIN A	2.0	*****					
IMMUNOGLOBULIN M	1.2	*****					
LUL PROTEIN	8.0	*****					
(PLANAR 28)							
TOTAL IMMUNOASSAY (G%)	5.4	*****					

THIS STUDY IS ABNORMAL FOR A PERSON OF THIS AGE AND SEX.

OF NOTE:

DESPITE NORMAL TOTAL PROTEIN THERE IS A SIGNIFICANTLY DECREASED LEVEL OF ALBUMIN WITH RELATIVE HYPERGLOBULINEMIA.

THERE IS A MARKEDLY ACTIVE INFLAMMATORY PROCESS. POSSIBLE ASSOCIATED CONDITIONS ARE:

- 1. RHEUMATOID ARTHRITIS
- 2. ULCERATIVE COLITIS
- 3. NEOPLASIA—PARTICULARLY A HYPERNEPHROMA
- 4. GRANULOCYTIC DISEASES
- 5. CHRONIC INFECTIONS
- 6. ETC.

ABNORMAL LEVELS OF IMMUNOGLOBULINS.

MAJOR THON STUDYING PROTEIN LEVELS, VALUES THIS LOW HAVE BEEN ASSOCIATED WITH BACTERIAL INFECTION AND HEMORRHAGUSIS.

Fig 10 Example of computer printout¹.

In a case of very low transferrin accompanied by only slight reduction in albumin (fig 10) the computer concluded that the low transferrin could be associated with bacterial infection, and suggested other possibilities. If the albumin were markedly reduced, a protein-losing syndrome would be more likely.

In addition to analysing such qualitative changes in the protein pattern, the computer can also be programmed to take account of the diagnostic significance of the degree of change in the levels of individual proteins.

If progress in this field is to continue, it is undoubtedly necessary to stimulate wider interest and to accumulate more data which must be technically reliable. This requires proper training of technicians, suitable instruments and reliable reagents. The clinician must be appraised of the clinical indications for making these measurements and of the significance of the results.