# COMPARATIVE STUDY OF TWO METHODS OF FIXING AND STAINING SERUM PROTEINS SEPARATED BY PAPER ELECTROPHORESIS

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The electrophoretic technique is widely used for the analysis of proteins in serum. Two methods that of Jencks *et al.*, and Connerty *et al.*, which involved different techniques of fixing and staining the proteins separated by paper electrophoresis were studied using both normal and pathological sera. It was found from this study that the method of Connerty *et al.*, is quick and gives equally accurate results and should be more widely adopted in clinical laboratory techniques.

Since its introduction by Tiselius in 1937, electrophoretic technique has undergone marked changes making it more simple and useful for accurate and rapid analysis of clinical samples. Like paper chromatography, it has earned an increasingly wide spread recognition as a research tool ( Luetscher 1941 ) that could be used for qualitative and quantitative analysis of complex mixtures and also for the assessment of diseases.

The staining methods for the separated proteins on filter paper were suggested by Gronwall (1952), Koiw *et al* (1952), Kunkel and Tiselius (1951), Dodson *et al* (1959), Jencks and associates (1955) and Connerty and others (1959). Most of these methods employ bromophenol blue as a dye and they vary widely both as regards the concentration of the dye and the staining time.

Two techniques, that of Jencks *et al* (1955) and Connerty *et al* (1959) are of interest. The latter is a rapid staining process while the former is a slow one. In order to determine whether the latter involving different fixing technique and staining time has any advantage over the former, the two techniques were studied and compared.

## METHODS

Sera from 30 subjects varying between 10 and 50 years of age were analysed. Out of 30 subjects 10 were patients and 20 were healthy subjects.

Serum was allowed to separate from fasting blood samples and the total proteins determined by the biuret method of Gornall and others (1949).

Whatmann No. 3 mm extra thick filter paper strips of the size 3.9 X 33.0 cms. were moistened with barbiturate buffer of pH 8.6 and ionic strength 0·075 M and were allowed to equilibrate for 1 hour in a horizontal electrophoretic tank. 0·02 ml. serum was spotted and duplicate strips were run for 16 hrs at 110 to 115 volts. One of the strips was then treated according to the method of Jencks *et al* (1955) and the other one according to the technique of Connerty and others (1959).

In the method of Jencks and associates (1955) proteins on the filter paper were fixed by drying in an oven at 110° C for half an hr. Later it was stained in a bath of 0·01 per cent aqueous solution of bromophenol blue for 16 hrs. and washed thrice with 2 per cent acetic acid followed by a final wash in 10 per cent acetic acid containing 2 per cent sodium acetate for exactly two min. The strip was blotted and dried in an oven at 110°C for 8 to 10 min.

The second strip was treated according to the method of Connerty *et al* (1959) wherein the proteins were fixed on the filter paper by placing it in a solution containing equal volumes of ethyl alchohol and ethyl ether for 10 min. It was then placed in a bath of a solution of equal volumes of ethyl alchohol and ethyl ether containing 10 per cent collodion for 10 min. The strip was air dried and then rinsed with 5 per cent acetic acid for 3 min. It was then stained in a bath of 0·5 per cent methanolic bromophenol blue for 30 min. Repeated washings of 6 min. each in three separate baths containing 5 per cent acetic acid were done. The strip was blotted and air dried and exposed to ammonia vapour.

The protein fractions separated by both the methods were deliniated visually and the strip was cut at the points of demarkation. Each fraction was cut into small peices and the dye was eluted by placing them in 0 01N sodium hydroxide solution for 30 min. The colour densities were read on Klett-Summerson Photoelectric Colorimeter which expressed as a percentage of that of the whole, thus getting the concentration of the different fractions. From the total protein concentration the concentration of the individual fractions were calculated.

## RESULTS AND DISCUSSION

Concentration of proteins both total and of the fractions were tabulated

The standard deviations were also calculated and are tabulated in Tables 1 and 2. It is obvious that there is no difference between the longer staining procedure and the shorter one.

TABLE No. 1

*Results of the fractionation of proteins in normal sera*

| | Name of Method | Albumin | alpha 1 | Globuline alpha 2 | Globuline Beta | Globuline Gamma |
|---|---|---|---|---|---|---|
| Mean | Method of Jencks *et al* | Gms.% 4·34 | Gms.% 0·405 | Gms.% 0·535 | Gms.% 0·79 | Gms.% 1·74 |
| | Method of Connerty *et al* | 4·31 | 0·411 | 0·546 | 0·78 | 1·75 |
| Standard Error | Method of Jencks *et al* | ±0·08616 | ±0·01131 | ±0·01414 | ±0·02458 | ±0·07269 |
| | Method of Connerty *et al* | ±0·1007 | ±0·01373 | ±0·01549 | ±0·02167 | ±0·07776 |
| t values | | 0·2265 | 0·3141 | 0·5251 | 0·2821 | 0·2971 |

TABLE 2

*Results of fractionation of protein in pathological sera*

| | Name of Method | Albumin | alpha 1 | Globuline alpha 2 | Globuline Beta | Globuline Gamma |
|---|---|---|---|---|---|---|
| Mean | Method of Jencks *et al* | Gms.% 3·21 | Gms.% 0·36 | Gms.% 0·57 | Gms.% 0·71 | Gms.% 1.586 |
| | Method of Connerty *et al* | 3·17 | 0·38 | 0·55 | 0·72 | 1·586 |
| Standard Error | Method of Jencks *et al* | ±0·1867 | ±0·03376 | ±0·03999 | ±0·05665 | ±0·1116 |
| | Method of Conaerty *et al* | ±0·1889 | ±0·03147 | ±0·03781 | ±0·05779 | ±0·1226 |
| t values | | 0·1506 | 0·4725 | 0·3635 | 0·1236 | 0·0061 |

The results obtained with the two methods show close agreement for both normal and pathological sera.

In both the methods bromophenol blue solution of different strength is used as a dye using different staining times. Though use of a dilute dye for a longer period facilitates the dye uptake reaching the equilibrium, the use of a concentrated dye for a shorter period achieves the same. The washing

technique as suggested in the method of Jencks and others (1955) helps in removing the dye almost completely from the background, but the more concentrated washing medium used in the method of Connerty and others (1959) does not remove the dye completely from the background and hence it increases the blank value which should be substracted from the absolute values of protein fraction. This rinsing error in the method of Connerty and others (1959) may be due to the use of more concentrated dye for a shorter period. The blue bands representing the proteins fractions, as obtained by the method of Jencks and others (1955) remain permanent but that obtained by the method of Connerty and associates (1959) are not stable and disappear very soon leaving more or less dark-greyish bands.

The use of sodium hydroxide as an elutent was found to be satisfactory though some authors like Dodson and others (1959) have used 4 per cent sodium carbonate in 50 per cent methanol in distilled water. Our observations are in close agreement with those of Connerty and others (1959) in that the strips processed according to their method requires nearly three hrs for complete elution.

Though the method of Connerty and others does not give stable blue bands of protein fraction it is quick and gives equally accurate results.

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