## Protein patterns in human parotid saliva

Attempts have been made by numerous investigators to separate and identify the various protein components in body fluids. From our past studies with parotid fluid using paper and agar gel electrophoresis, it was apparent that a better resolution was needed. Disc electrophoresis (developed by ORNSTEIN AND DAVIS[1]) using small columns of acrylamide gel, appears to be the best method yet devised for protein separation. This system allows the operator to use micro amounts, and complete a run in about 50 min.

Investigators have found that numerous systemic diseases give a characteristic blood serum protein pattern. HAMMACK *et al.*[2] show this in their work with paraproteinemias, as did KOCHWA *et al.*[3] and ZINGALE *et al.*[4]. The latter were quick to describe disc electrophoresis as a valuable procedure for the diagnosis of macroglobulinemia.

NARAYAN *et al.*[5] have modified the disc procedure for use in animal serum protein studies. The experiments in this laboratory are aimed at studying pathology of the parotid gland and oral region in regard to protein content of the parotid fluid. To identify the pathological protein pattern a non-pathological pattern must first be established. Using disc electrophoresis, with some modifications, we have attempted to separate parotid proteins and group them as either lipoproteins, glycoproteins, or unconjugated proteins. We are also attempting to identify normal parotid protein patterns.

*Methods and materials*

Seventy-nine healthy males with an average age of 24 years were used for the study. Their medical and oral history had no apparent outstanding differences. The parotid flow was stimulated with lemon juice and collected via a modified acrylic Carlson–Crittenden apparatus. Fifteen to 30 ml were collected at a time, and the fluid was dialyzed against either 20 volumes of physiologic saline (this fluid was also used for amylase studies) or 20 volumes of distilled $H_2O$. Both solutions were held at 4° and dialysis continued for 24 h. Non-dialyzed parotid fluid inhibits the polymerization of the anti-convection sample gel. NARAYAN *et al.*[5] noted this problem in working with fresh blood sera. Although it is suspected that some proteins are lost in this process, DAWES[6] states that at a pH of 8.6 they are cationic fractions. We are separating only anionic proteins. The samples were then lyophilized and reconstituted at a dilution of 0.4 mg/0.03 ml $H_2O$. An amount of 0.06 ml of sample was placed in each column. Standard 7.5 % acrylamide gel was used as the supporting medium in 5 mm i.d. tubes. A direct current was applied at a constant 5 mA/cell for about 50–65 min, using a tris-glycine buffer, pH 8.3, ionic strength 0.01. A bromphenol blue tracking dye was added. The mobility of this dye exceeds that of the fastest moving protein.

The stains used in this study were Edward Gurr. The protein bands were stained with amido black, 0.55 g in 100 ml of 15 % HAC. The staining time is not under 1 h.

To detect lipoproteins, a solution of 40 % EtOH saturated with Sudan black was used. Staining time was 6 h on a shaker. First the gel slugs must be fixed in 15 % HAC for 1 h.

P.A.S. (Periodic Acid Schiff) and Alcian blue 0.2 % solution in 15 % HAC, were used to detect glycoprotein. Alcian blue stains acid groups on the carbohydrates.

It has been suggested by CHANG *et al.*[7] that the mobility of each band can be expressed as a percentage of the mobility of the added tracking dye.

The tracking dye is lost during the de-staining procedures, and it is the practice in this laboratory to sever the gel slug at the position of the tracking dye upon completion of the run, and prior to de-staining. This gives a consistent point of reference by which the relative mobility of the protein band may be compared, and alleviates a remote chance of mistaking a swiftly moving protein for residual tracking dye.

To calculate the $R_M$ (relative mobility), the total distance traveled by the tracking dye is divided into the distance traveled by the farthest edge of the protein band. This gives a method similar to the $R_F$ of paper chromatography for identifying band position. Caution must always be taken to immerse the gel column in the same solution (15 % HAC) because the gel slug will not be the same size in a 40 % EtOH solution as it would be in an $H_2O$ solution. When special stains are used requiring different solvents, the gel slug is placed in the 15 % HAC before attempting to measure the $R_F$, even though the shrinkage is probably uniform.

*Results and discussion*

Tube A in Fig. 1 contains a gel slug run under the above prementioned conditions and stained with amido black. In this particular healthy patient we were able to discern 13 protein bands. This is about the average number seen — usually 12–14 bands are noted with our methods. Consistencies observed in 79 specimens are as follows: in 75 samples at least 3 fine bands with $R_M$'s between 0.008–0.098 are found. In 5 cases there has been a splitting of band 3 into 2 bands. With modified technique, band 3 may prove to be 2 different proteins.

In the area immediately above band 4A another band has been noted in 26 of the 79 samples. This band appears fused with band 4A, but in 4 of the cases a definite separation was noted indicating this slower band is not an artifact. The range of the $R_M$ was 0.172–0.271 for these 26 bands.

The $R_M$ of the darkest area present in parotid fluid ranges from 0.187 to 0.308. Band 4A of the amido black stained gel A in Fig. 1 approximates the $R_M$ of band 1B of the Sudan black stained gel B band and 1D of the P.A.S. stained gel D. Because of the intense background staining the band in gel D is difficult to picture with the camera. The slight difference in $R_M$ may indicate that band 4A is a heterogeneous band with both glycoprotein and lipoprotein present. They are so closely related in relative mobility that they stain as one or are masked when the amido black stain is used.

Even though in 79 samples there was a variation in the relative mobility of band 4A, individual samples run in the same environment, *i.e.*, run at the same time in the same unit, have shown an average per cent deviation from the mean no higher than 5.3 % and as low as 1.7 %. When samples are not run in the same environment the utmost care must be taken with control procedures.

Bands 5A, 6A, 7A, and 8A appeared in 49 of the 79 samples. At least 3 of these 4 bands appeared in all but 9 samples. This area of the pattern is often diffused and this could account for the finding of only 2 bands in 9 of the samples.

The locale in which bands 9A and 10A appear usually contain 3 to 5 bands. If additional bands are observed, it is in this area. They are diffused and never darker than band 9A in Fig. 1.

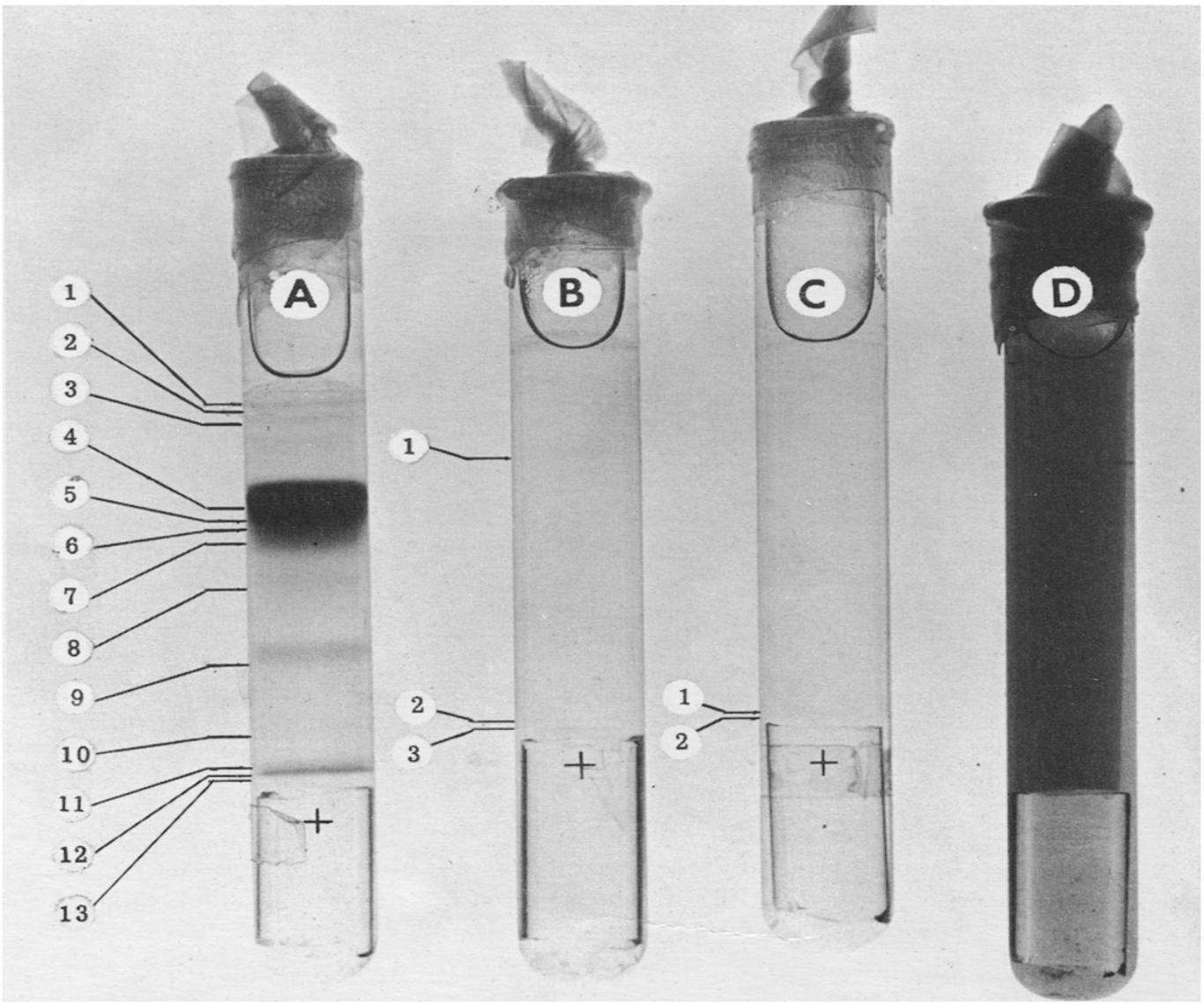


Fig. 1. Stained parotid proteins in acrylamide gel. All 4 tubes contained parotid fluid from the same sample. These were run concurrently and stained as follows: Tube A (amido black), Tube B (Sudan black), Tube C (Alcian blue) and Tube D (P.A.S.).

Band 11A was discernible in 34 of the 79 samples. Band 12A is sharper than band 11A and appears in all 79 samples. Band 13A is a faint, fast moving protein that appeared 19 times, probably undetected because of its low concentration in parotid fluid.

Bands 11A, 12A, and 13A, when especially stained with Sudan black and Alcian blue indicate conjugated proteins (see Table I). Band 11A, $R_M$ 0.943, compares closely with the 0.947 $R_M$ of the Sudan black band 2B, thus indicating a probable lipoprotein. Band 12A, $R_M$ 0.959, compares closely with band 1C of Alcian blue, $R_M$ 0.961, indicating a probable glycoprotein.

Band 13A, $R_M$ 0.981, approximates the 0.972 of band 3B and 0.974 of band 2C of the Sudan black (tube B) and the Alcian blue (tube C) respectively, indicating the possibility of a glycolipoprotein.

When P.A.S. stain is used on these gel columns the area of bands 11A, 12A, 13A shows a positively stained portion but the result is too diffused to demonstrate bands. Further demarcation was obtained with Alcian blue.

TABLE I

CHART OF RELATIVE MOBILITY VALUES

| *Tube A* *Amido Black* | | *Tube B* *Sudan Black* | | *Tube C* *Alcian Blue* | | *Tube D* *P.A.S.* | |
|---|---|---|---|---|---|---|---|
| *Band* | $R_M$ | *Band* | $R_M$ | *Band* | $R_M$ | *Band* | $R_M$ |
| 1A | 0.034 | | | | | | |
| 2A | 0.048 | | | | | | |
| 3A | 0.080 | | | | | | |
| 4A | 0.290 | 1B | 0.277 | | | 1D | 0.298 |
| 5A | 0.322 | | | | | | |
| 6A | 0.372 | | | | | | |
| 7A | 0.384 | | | | | | |
| 8A | 0.497 | | | | | | |
| 9A | 0.685 | | | | | | |
| 10A | 0.865 | | | | | | |
| 11A | 0.943 | 2B | 0.947 | | | | |
| 12A | 0.959 | | | 1C | 0.961 | | Diffused |
| 13A | 0.981 | 3B | 0.972 | 2C | 0.974 | | |

The bands are numbered in order from the top of the column with the $R_M$ calculated by dividing the distance the band traveled by the tracking dye distance. These tubes are from the same sample and run at the same time.

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