## An Electrophoresis Apparatus for analysing Several Sera Simultaneously

A systematic study, such as we contemplate, on the influence of disease on the electrophoresis pattern of serum, requires observations on large numbers of sera. Such an investigation is laborious and time-consuming when the available techniques and apparatus are utilized, because only a very limited number of experiments can be completed in one day. We have, therefore, devised a method whereby two electrophoretic analyses can be made simultaneously using the Tiselius apparatus with a standard central section.

The apparatus is illustrated in Fig. 1. The electrode vessels are connected on to 'Perspex' adaptors (*A* and *B*) and the standard central section of the electrophoresis cell held between them. Prior to assembly, the ground surfaces of the 'Perspex' adaptors as well as those of the electrode cell are well greased, and thin 'Cellophane' membranes are then clamped between the sections in the position indicated ($M_1$ and $M_2$). The protein solutions to be analysed are held between the membranes, and the rest of the apparatus filled with buffer and saturated potassium chloride in the usual way. The dimensions of the adaptors are such that they fit the Hilger electrophoresis bath and cell holder.

A *p*H for electrophoresis must be selected where all the components present migrate towards the bottom membrane. In the case of serum, this is achieved at *p*H values above neutrality.

In Fig. 2 are shown electrophoresis diagrams of two samples of the same bovine serum obtained simultaneously using the two limbs of the standard electrophoresis cell. On inspection of the diagrams it will be noticed that they are identical. The reason for the markedly enhanced δ-effect visible at the beginning of the electrophoresis diagram is, as yet, unexplained. In the diagram of the same serum obtained with the standard apparatus, the δ-effect was small.

Electrosmotic effects do exist; but apparently they have no effect on the electrophoresis diagram. Mobility measurements calculated from electrophoresis diagrams obtained in the modified apparatus described are likely to be effected by the electrosmotic effects, but the samples studied simultaneously are comparable.

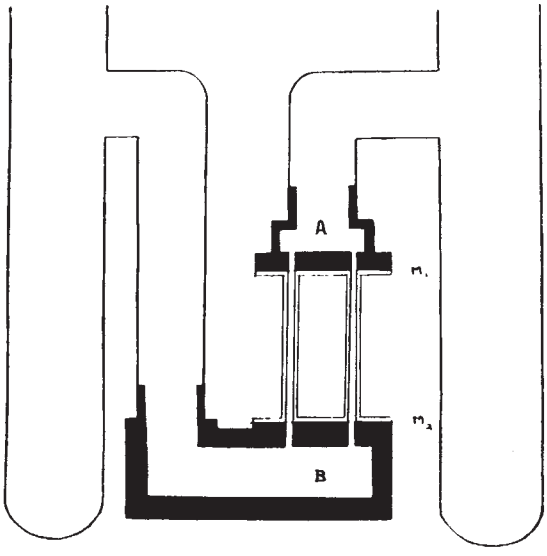


Fig. 1. Assembled electrophoresis cell. Shaded parts (*A* and *B*) are made of 'Perspex'. $M_1$ and $M_2$ are 'Cellophane' membranes

Fig. 2

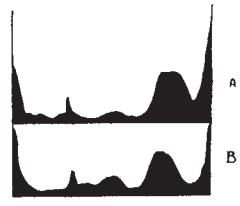


Fig. 3

Fig. 2. Electrophoresis diagrams of two samples of the same bovine serum obtained simultaneously

Fig. 3. Electrophoresis diagrams of normal serum (*A*) and serum from a chronic nephritic (*B*). They were obtained simultaneously by the use of the modified cell

The technique described points the way to a means of mass electrophoresis of sera, as there is no theoretical limit to the number of compartments which can be built into the analytical section of the cell.

A further advantage of this type of cell is that exact comparisons can be made between sera obtained from different sources. This advantage is clearly brought out in Fig. 3, which shows the difference between the serum from a normal and a chronic nephritic subject. The nephritic serum albumin has a mobility slightly less than that of the normal albumin. The α-globulin has the same mobility in both sera, and the β-globulin in the normal serum has, in turn, a lower migration-rate than the corresponding globulin in nephritic serum.

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## Isolation of a Crystalline Disaccharide, Hyalobiuronic Acid, from Hyaluronic Acid

The structure of hyaluronic acid, an important constituent of the 'ground substance', has been the subject of intensive study in recent years. Applications of conventional methylation and periodate consumption methods to the intact polysaccharide[1,2] have indicated that such methods will not yield substantial evidence of structure. The isolation and clarification of structure of crystalline oligosaccharides derived from hyaluronic acid is the most rigorous method of studying the structural details of this physiologically important substance. Such well-defined compounds are also required as models for establishing the specificity of the enzyme systems acting on hyaluronic acid.

The present communication is concerned with the isolation and characterization of a crystalline aldobiuronic acid from sodium hyaluronate in relatively high yield, following degradation by a combination of enzymic and mild acid hydrolysis. Sodium hyaluronate (analysis[3]: nitrogen, 3·78; uronic acid, 44·0; hexosamine, 38·8; sulphate, less than 0·5) was hydrolysed with purified testicular hyaluronidase

(600 turbidity reducing units per mgm.) until the increase in reducing sugar[4] was equal to 0·35 mgm. of glucose equivalent per mgm. of substrate. The small precipitate obtained on addition of two volumes of ethanol was removed, and the filtrate was then concentrated *in vacuo* to remove the ethanol. Addition of 4 volumes of absolute ethanol resulted in separation of a syrup, which was hardened with ethanol. The colourless, amorphous product was dried over calcium chloride and gave the following analysis: nitrogen, 2·81; uronic acid, 39·7; hexosamine, 37·9; reducing sugar (as glucose), 30·9. The yield from 1·91 gm. of sodium hyaluronate was 1·84 gm. A solution of 1·00 gm. of this product in 20 ml. of 1·0 *N* sulphuric acid was heated for 3 hr. at 99° in a sealed tube, then cooled and neutralized to *p*H 5·0 with saturated barium hydroxide. After removal of the precipitate, the amber solution was concentrated to 7 ml. and chilled, whereupon 300 mgm. of colourless material, appearing as rectangular, long prisms under the microscope, was deposited. Reheating the filtrate and washings with sulphuric acid, followed by neutralization and concentration, yielded an additional 130 mgm. Reprecipitation from 0·2 *N* hydrochloric acid solution with 1·0 *N* sodium carbonate gave homogeneous, long, rectangular prisms, weighing 232 mgm. after drying *in vacuo* over calcium chloride. The analysis of this material is presented in the accompanying table, with the values calculated for a disaccharide containing one residue of glucosamine and one of glucuronic acid.

ANALYSIS OF HYALOBIURONIC ACID

| | Calc. for $C_{13}H_{21}O_{11}N$ | Found |
|---|---|---|
| Carbon | 40·56 | 40·01 |
| Hydrogen | 5·96 | 6·02 |
| Nitrogen (Dumas) | 3·94 | 3·99 |
| Amino nitrogen (van Slyke) | 3·94 | 3·96 |
| Hexosamine | 50·5 | 49·0 |
| Uronic acid (gasometric) | 55·2 | 54·3 |
| Reducing sugar, ferricyanide[4] | | 68·0 |
| Reducing sugar, hypoiodite[5] | | 61·0 |
| $[\alpha, 24°, D]$ (2·00 per cent in 0·10 *N* hydrochloric acid) | | +31° |

Potentiometric titration indicated a substance with a $pK_1'$ of 2·6 and a $pK_2'$ of 7·1. On a micro hot-stage, the substance slowly decomposed without melting, beginning to darken at about 200°. The material was insoluble in glacial acetic acid, ethanol, methanol and pyridine, and almost insoluble in water. It was sparingly soluble in hot water, dilute hydrochloric acid, and dilute sodium bicarbonate. On a paper chromatogram developed by the ascending method with butanol – acetic acid – water (38 : 12 : 50) and then sprayed with *p*-dimethylaminobenzaldehyde, the material migrated with an $R_F$ of 0·13, compared with 0·29 for glucosamine, and 0·43 for N-acetylglucosamine. The colour was identical with that given by glucosamine. The direct Elson–Morgan reaction (without preliminary hydrolysis) gave a colour value equivalent to 29 per cent of glucosamine by weight. The glucosamine residue, therefore, has a colour yield in this reaction which is only about 60 per cent of that of free glucosamine on a molar basis.

Despite the constancy of the chemical properties of all fractions examined in the course of these experiments, and the homogeneity with respect to crystal habit of the fraction described here, the analysis of this fraction by a solubility method gave results not consistent with the presence of a single species. This observation may be the result of an artefact of the analytical method, or of physical or chemical transformations which frequently occur with carbohydrate compounds. On the other hand, it may reflect the presence of two species with a structural difference due to the structure of the original polysaccharide. Further study will be required to clarify this point.

In any event, the isolated material, for which we propose the name 'hyalobiuronic acid', has the structure of a glucuronido–glucosamine with the free reducing group in the glucosamine residue. The insolubility in water must be attributed in part to the existence of the material in zwitterionic form. The stability to acid hydrolysis indicates that the glucuronic acid residue is present as a pyranoside. It is perhaps worth while to point out that hyalobiuronic acid will require re-acetylation of its amino group before a model can be obtained which will be suitable for comparison with the products obtained by enzymic hydrolysis of hyaluronic acid.

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## A Brown Discoloration of Pig Fat and Vitamin E Deficiency

THE consumption of vitamin E-deficient diets rich in highly unsaturated fatty acids, by chicks and by rats, is followed by the appearance of a yellow-brown coloration of the adipose tissue[1,2]. This pigmentation can be prevented by feeding a supplement of α-tocopherol acetate[3].

In an experiment carried out at the Agricultural Research Institute (N.I.), six female pigs were given a vitamin E-deficient diet containing 5 per cent dried skim milk powder, 5 per cent dried yeast, 5 per cent white fish meal, 5 per cent liver meal, 15 per cent palm kernel meal (ext.) and 65 per cent cassava meal, with a mineral supplement of salt and limestone; the vitamin E content was less than 0·1 mgm./100 gm. In addition, two of the pigs (group *A*) received 1 oz. cod-liver oil, as a vitamin supplement, once per week from weaning until they reached 100 lb. live-weight, two pigs (group *B*) received cod-liver oil throughout, at the rate of 5 per cent of their meal ration, while the remaining pigs (group *C*) received cod-liver oil at the 5 per cent level, together with 50 mgm. *dl*-α-tocopherol acetate a head each day. The meal was fed individually to all pigs, as a wet mash, on a slightly restricted scale of feeding[4]; in groups *B* and *C* the cod-liver oil was added directly to each morning feed, and in group *C* the tocopherol