Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 20, Number 2, April, 1945.

Printed in the Union of South Africa by the Government Printer, Pretoria.

An Electrophoresis Apparatus for the Rapid Routine Analysis of Sera and Other Protein Solutions.

By ALFRED POLSON, Section of Virus Diseases, Onderstepoort.

In previous reports [Polson (1940, 1941)], an electrophoresis apparatus has been described which was used for measuring mobilities of horse sickness virus and its neutralising antibodies in low concentration. The method depended on the analysis of the fluid in the limb in which the virus or antibody was ascending. With the apparatus then used only one experiment a day was possible as the permissible maximum voltage gradient which could be employed without causing convection currents was small.

Several improvements have subsequently been introduced into the method of electrophoresis. These are briefly:—

(1) *Low temperature of Electrophoresis.*—In order to use higher voltage gradients and thus shorten the time of electrophoresis the apparatus was mounted in a waterbath at low temperature as recommended by Tiselius (1937). The actual temperature at which the experiments were performed was 2° C. which was slightly lower than the temperature used by Tiselius i.e. 4° C. The temperature of the bath was controlled by means of a household freezing unit in conjunction with a contact thermometer and relay. The refrigerant methyl chloride passed through copper coils in the water bath.

Method for removing the electrophoresis cell out of the water bath without disturbing the fluid in the cell.—To avoid rinsing the electrophoresis cell between the different experimental runs at the same pH, the cell-holder was attached to an X-ray tube-holder by means of which it could be moved to any desired position. With this holder the electrophoresis cell could be lifted out of the waterbath without disturbing the fluid in it. In Fig. 1 a photograph of the cell attached to the holder is given just before it was lowered into the waterbath. After completion of an experiment the " spent " serum was run out and an equal volume of fresh buffer was inserted. The cell was again lowered into the bath and when temperature equilibrium was attained, fresh diluted serum was let in through the stopcock by means of a manometer arrangement.

The whole process of taking the cell out of the bath, removing the spent serum and inserting fresh serum can be completed in less than 10 minutes. This method has the advantage that the use of buffer salts is reduced to a minimum; at least eight successive sera can be analyzed before it is necessary to change the salt solutions in the electrode vessels.

An additional advantage is that both the ascending and descending columns can be photographed by merely shifting the horizontal rod to which the cell-holder is attached.

Rectification of boundary anomalies.—In electrophoresis it is very essential to have sharp initial boundaries or interfaces between the protein and the solvent in the U-tube. To obtain this the following device was used. A rack pinion of a microscope provided with a clamp to grip a pipette was screwed to the holder of the cell above the open ends of the U-tube. With this arrangements a very fine pipette could be lowered into the limbs of the U-tube and fluid withdrawn slowly at the imperfect interface protein-buffer. By doing this a very sharp interface could be formed.

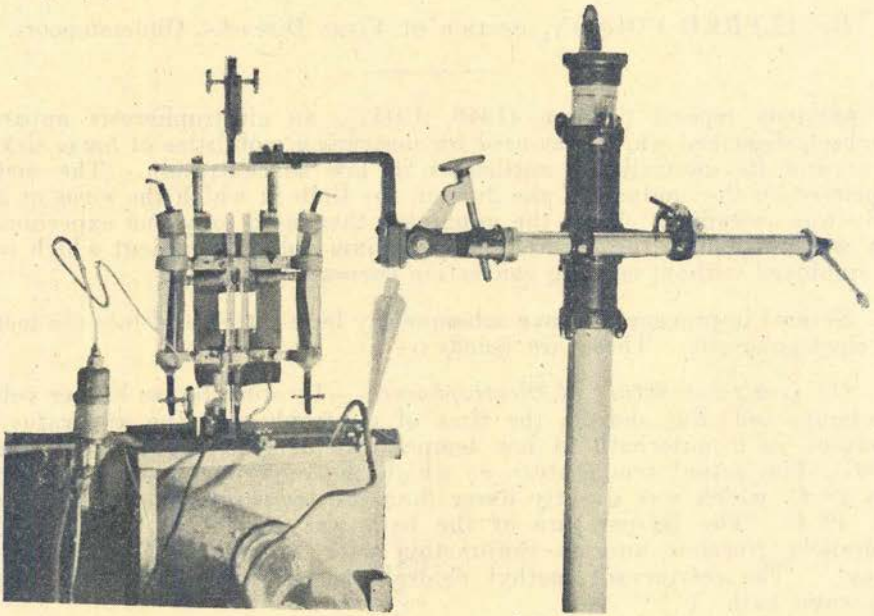

Fig. 1.—Electrophoresis cell fixed on to X-ray tube-holder. Note the rack and pinion of the microscope above the open ends of the U-tube.

(4) *Registation of Migrating Components.*—To register the migration of the protein components, the Lamm scale method, [Lamm (1928, 1937)] was used. This method, although rather laborious, gives far more accurate results than the other methods of observing the boundaries such as the Philpot, Svensson, Anderson, Longsworth, or " schlieren " methods c.f. Svedberg and Pedersen (1940). A further advantage of the scale method is that fewer photographic plates are required. During routine analysis of sera it was possible to make 20 photographs of the scale on one 9×12 cm. photographic plate.

(5) *Identification of components in the electrophoresis diagrams:*— During electrophoretic analysis of protein mixtures it is often necessary to determine which peak in the diagram represents a certain fraction. To do this the following procedure was followed.

An electrophoresis run was made at the pH where the greatest difference in mobilities amongst the various components exists. This pH must be within the stability regions of the different protein components. A photograph was taken at the end of the run. A fine pipette with its point bent at right angles to the pipette was lowered into the limb of the U-tube by means of the rack and pinion screwed to the cell-holder until the first component in the tube was reached. The manometer negative pressure connected to the pipette was released and 0·25 c.c. of fluid was sucked in slowly. The pipette was then emptied in a serological tube, and a photograph of the scale then taken. This process was repeated until the whole ascending column was cut up in equal sections. Care was taken to have all the samples taken in the same position in the tube. The electrophoresis was then reversed and samples and photographs taken of the descending column.

TABLE 1.

Ascending Buffer:—0·0133 *M phosphate* 0·05 *M NaCl pH* 6·2.

Sample No.—	½	¼	⅛	1/16	1/32	1/64
1.....	0	0	—	—	—	—
2.....	+	+	0	0	—	—
3.....	+	+	+	0	0	—
4.....	+	+	+	0	0	0
5.....	+	+	+	+	0	0
6.....	+	+	+	+	0	0
7.....	+	+	+	+	0	0
8.....	+	+	+	+	0	0
9.....	+	+	+	+	0	0
10.....	+	+	+	+	0	0
11.....	+	+	+	+	0	0

Descending

Sample No.—	½	¼	⅛	1/16	1/32	1/64
1.....	0	0	—	—	—	—
2.....	0	0	—	—	—	—
3.....	0	0	—	—	—	—
4.....	0	0	—	—	—	—
5.....	+	+	+	0	0	—
6.....	+	+	+	0	0	—
7.....	+	+	+	0	0	—
8.....	+	+	+	+	0	—
9.....	+	+	+	+	0	—
10.....	+	+	+	+	0	—
11.....	+	+	+	+	0	—

The activities of the samples were then determined in their particular way and compared with diagrams obtained. By analyzing both the ascending and descending columns the activity can be located to a definite component. This process of analysis is illustrated below in an electrophoretic analysis of cobra venom. It was required to analyze cobra venom electrophoretically

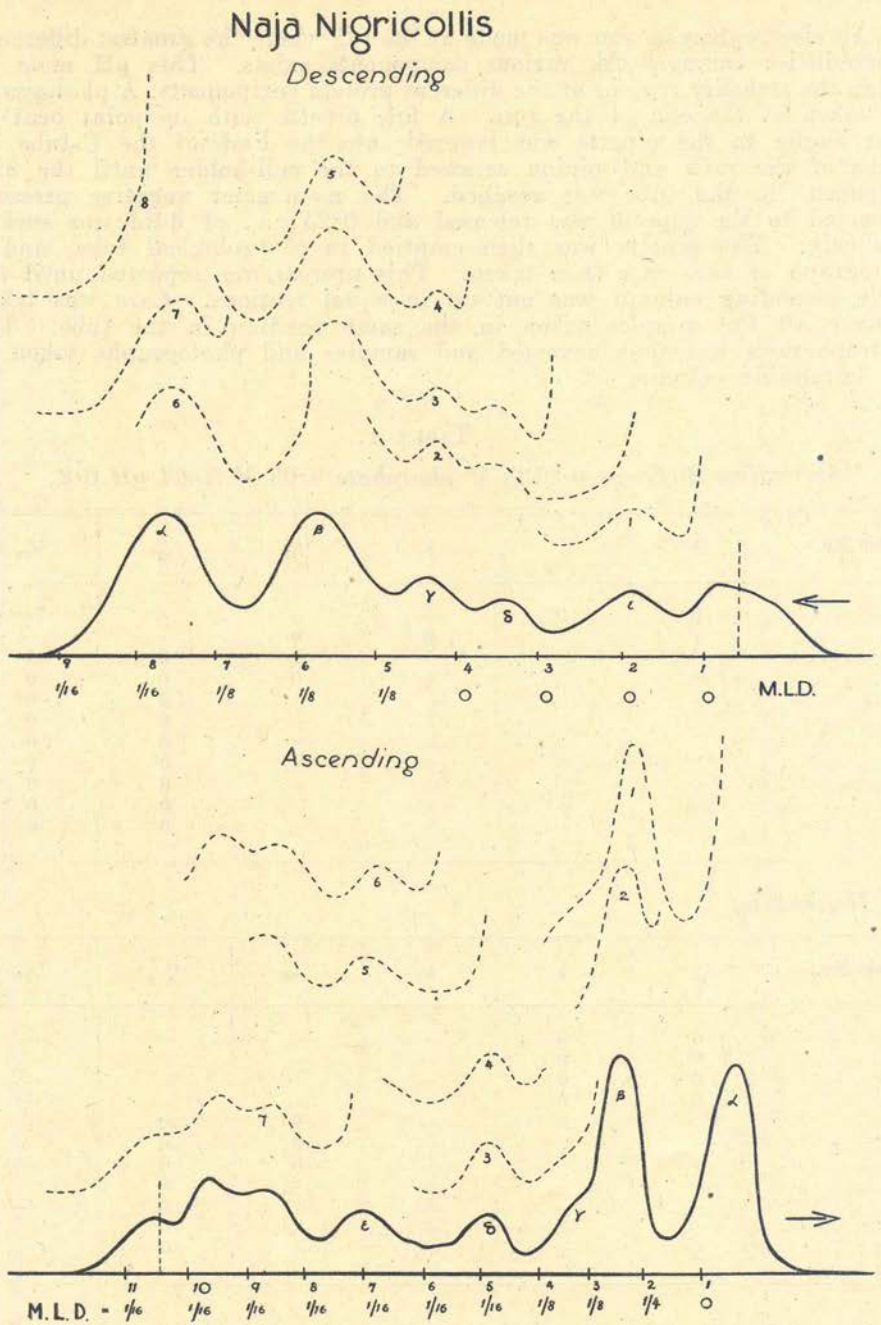

Fig. 2.—Electrophoresis diagrams of the venom of *Naja nigricollis* (spitting cobra). The dotted curves are the diagrams obtained after the different samples have been removed. The full lines are the diagrams of the venom in the descending and ascending columns. The vertical dotted lines are the initial positions of the boundaries. The arrows indicate the direction of migration. This figure was taken from a forthcoming article on cobra venom by Polson and Haig.

and to determine from the diagram obtained which component was responsible for its lethal action. Good separation between the components occurred when the experiments were run at pH 6·2 and with a voltage gradient of 6·5 volt/cm. The duration of the electrophoresis was 120 minutes. In figure 2 are given the curves obtained for the ascending and descending columns together with the curves obtained after each sample was withdrawn from the tube. In Table 1 the activities of the different samples are given as determined by the intravenous injection of mice.

It will be observed when comparing the results in Table 1 with Fig. 2 that the activities can be ascribed to component β (mobility $6{\cdot}2 \times 10^{-5}$ cm^2/sec/volt) in the diagram on the ascending as well as on the descending side.

It must be noted here that the peak of the lethal component in the diagram does not coincide with the maximum lethal activity of the samples as the inflection point of the peak represents the position of 50 per cent. concentration. Full activity will first be shown by samples which are taken in the region where no spreading effects of the lethal component exist.

Summary.

An electrophoresis apparatus is described which was found suitable for the rapid analysis of sera and other protein mixtures. A method for identification of protein components in the migrating column is given.

Acknowledgments.

I wish to express my sincere gratitude to Dr. R. A. Alexander of this institute and to the Director, Dr. P. J. du Toit for their very encouraging interest in this work; also to Dr. J. H. Mason of the S. A. Institute for Medical Research, Johannesburg for the donation of the snake venom used in this work.

LITERATURE.

LAMM (1928). Zur Bestimmung von Konzentrations—graduenten mittels gekrümmter Lichtstrahlen. *Z. physik Chem. A.*, Vol. 138, p. 313.

LAMM (1937). Measurements of Concentration Gradients in Sedimentation and Diffusion by Refraction Methods. Solubility Properties of Potato Starch. *Nova Acta Reg. Soc. Scient. Upsal.*, Ser. IV, Vol. 10, No. 6.

POLSON (1940). Electrophoresis of Animal Viruses and their Neutralizing Antibodies in Low Concentrations. *Nature*, Vol. 145, p. 27.

POLSON (1941). The Electrophoresis of the Neurotropic Virus of Horsesickness and its Neutralizing Antibodies in Low Concentration *Onderstepoort Jl.*, Vol. 16, p. 51.

SVEDBERG AND PEDERSEN (1940). The Ultracentrifuge. Oxford.

TISELIUS (1937). A new apparatus for electrophoretic analysis of colloidal mixtures. *Trans. Farad. Soc.*, Vol. 33, p. 524.