APPLICATIONS OF ELECTROPHORESIS TECHNIQUE IN FORENSIC SCIENCE

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THE forensic scientist is frequently called upon to detect and estimate traces of drugs and poisons of animal, plant or synthetic origin in a great variety of biological material. A large number of tests are published in scientific literature for the detection and estimation of many substances. Most of these chemical and biological tests are non-specific and hence they are of little use when employed directly on the material. It is, therefore, necessary to employ techniques which will resolve complex mixtures into individual components on which the chemical and biological tests can be safely applied to express a correct and definite opinion. Moreover, the quantity of material available for such examination is usually very small. Techniques of electrophoresis and paper chromatography discovered by Tiselius,[1] and Martin and Synge[2] respectively are invaluable tools in a forensic science laboratory for the separation of individual components in complex mixtures, particularly when the quantity available is very small. It is proposed to deal with the technique of electrophoresis and its applications in this paper.

Tiselius used boundary electrophoresis as a means of separating the protein components of plasma as early as 1937. A large number of workers have modified and used this technique in the analysis of complex protein mixtures and other compounds capable of possessing a charge in an aqueous buffer.

The apparatus necessary for boundary electrophoresis is complex and expensive. Hence paper electrophoresis, which is a very simple technique, first employed by Wieland and Fischer,[3] in 1949, has been extensively used in recent years for the separation of gonadotrophin in urine,[4] Thyroglobulin in serum,[5] muscle extracts,[6] Isoaglutinins[7] and other materials. Mackay[8] and Cooper[9] have carried out the analysis of human serum by this technique. It is not necessary to describe the electrophoresis apparatus and the experimental procedure as they are generally well-known and are in use in many laboratories in India.

The curves are obtained by scanning the papers with a suitable Densitometer. The areas under the Densitometer curves are measured with a Planimeter and the amount of each component is expressed as a percentage of the total area. This percentage will be proportional to the amount of protein provided the technique is standardized very carefully.

This technique has been employed to study the electrophoretic patterns of the blood sera from the different species. The data obtained by Mickinlay and Farmilo[10] for the sera of Horse, Rat, Dog, Sheep, Buffalo, Rabbit, Cow, Pig and Guineapig are given in Tables I and II. If a number of sera are separated on the same sheet of paper it would be possible to obtain a direct comparison under identical conditions. The individual pattern for these species is quite characteristic. The albumin fraction of most species moved at about the same rate in most instances but the albumin fraction of Dog sera was always more mobile than that of any other species studied. The albumin fraction of Guineapig and Rat sera moved at slower rates than the albumin from other species. The data presented here indicate that there are considerable

TABLE I

	Albumin	Globulines								
		α_1	α_2	α_3	β	β_1	β_2	γ	γ_1	γ_2
Horse	44·84	2·34	8·13	..	11·89	..	..	34·74	..	..
Rat	46·10	4·52	10·83	..	20·15	..	..	18·41	..	..
Dog	51·41	4·52	4·52	5·02	..	6·17	18·41	..	..	9·95
Sheep	37·40	2·29	3·16	4·36	..	7·27	9·22	..	36·30	..
Buffalo	32·35	..	26·03	..	8·48	..	..	33·14	..	..
Rabbit	52·30	3·66	9·12	3·98	..	13·10	3·34	14·5	..	..
Cow	46·50	..	13·60	..	..	9·30	..	..	17·80	12·80
Pig	31·60	2·06	16·82	9·05	..	..	17·00	..	..	23·47
Guineapig	58·58	4·11	11·46	..	11·46	..	..	14·39	..	..

TABLE II

Relative mobilities of serum protein components expressed as a fraction of the distance moved by the albumin of the same serum

	Albumin	α_1	α_2	α_3	β_1	β_2	γ_1	γ_2
Horse ..	1·00	0·79	0·66	..	..	0·47	0·23	0·05
Rat ..	1·00	0·80	..	0·65	..	0·40	..	0·06
Dog ..	1·00	0·90	0·78	0·08	0·56	..	0·34	0·09
Sheep ..	1·00	0·84	0·76	0·62	0·49	..	0·34	0·20
Buffalo ..	1·00	..	0·74	..	0·54	..	0·23	..
Rabbit ..	1·00	0·85	0·73	0·65	0·56	0·47	0·21	..
Cow ..	1·00	0·86	0·72	..	0·51	..	0·36	0·13
Pig ..	1·00	0·87	0·63	..	..	0·39	..	0·11
Guineapig ..	1·00	0·85	0·68	..	..	0·39	..	0·11

differences in the electrophoretic patterns of sera from different species of animals and this technique can be used for characterising animals of different species. Connel[11] has demonstrated that the muscle extract from 20 different species of fish gave characteristic electrophoretic patterns. From the medico-legal point of view this technique can be applied for the identification of the species from a blood stain which should be extracted with normal saline before running the electrophoresis.

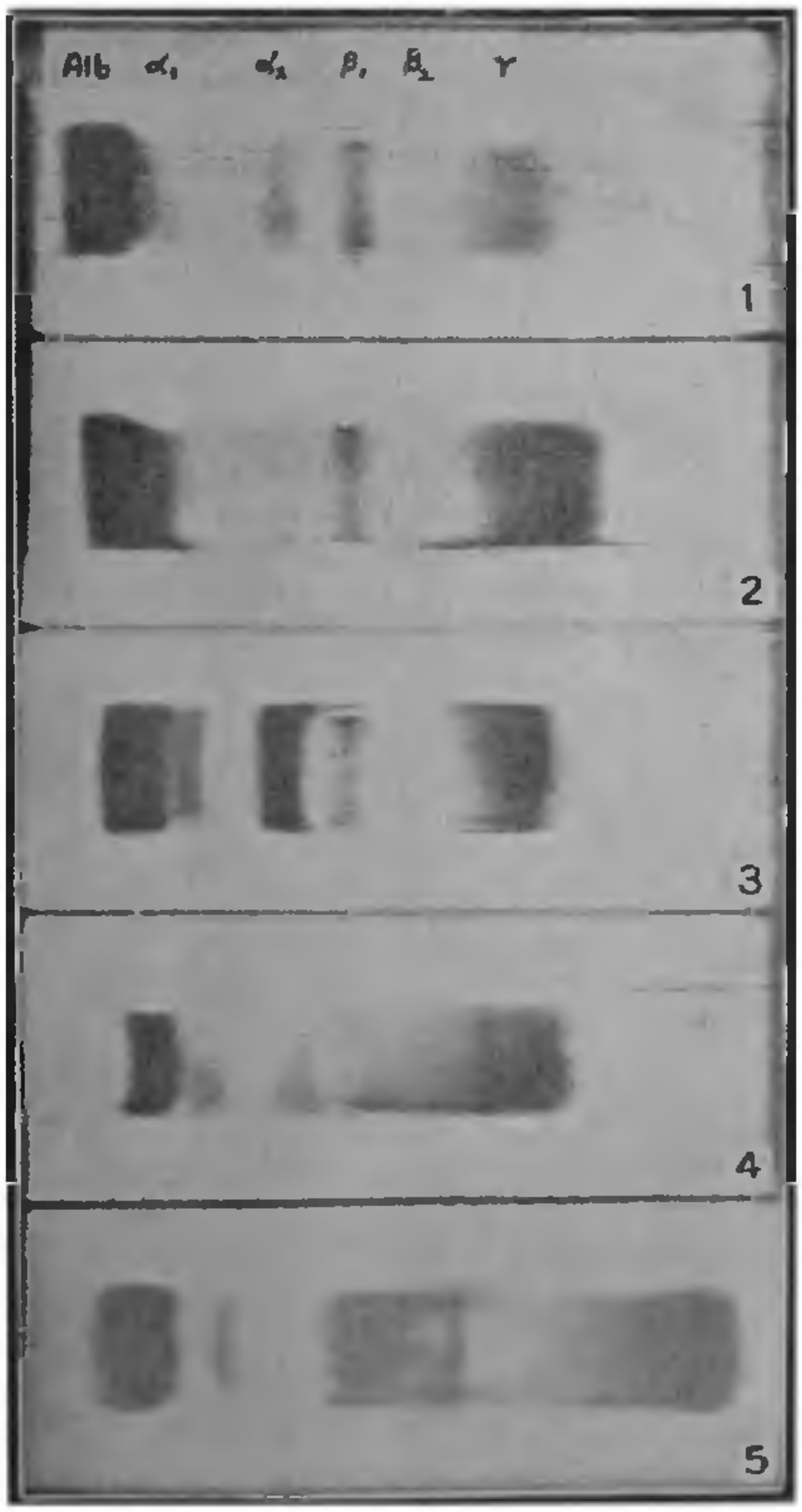

FIG. 1. (1) Normal serum. (2) Cirrhosis. (3) Pulmonary Tuberculosis. (4) Nephritis. (5) Cancer (Stomach).

In recent years, this technique has been used extensively for diagnostic purposes in pathological laboratories attached to hospitals. The characteristic electrophoretic patterns for a number of specific diseases have been worked out by Giri[12] and these have been reproduced in Fig. 1. If a suspect involved in a criminal case happens to be suffering from a particular disease resulting in a characteristic electrophoretic pattern of the blood, it may be possible in rare cases to state that the blood found at the scene of the crime belongs to that particular individual, if there is other circumstantial evidence in favour of this hypothesis.

In order to control the International Drug Traffic, the geographical region of opium in the illicit trade must be known so that the supplies of the drug may be cut off at the source. To this end, the Economic and Social Council of the United Nations in 1948-49 authorised research into devising chemical methods of identifying opium and invited member Governments to participate in such a programme and to provide authentic samples for opium research. The technique of paper electrophoresis has been applied for the detection of the country of origin of opium. Opium samples from different countries were ground in a mortar in 10% acetic acid and filtered. A portion of the clear filtrate was applied to the paper and electrophoresis was carried out in the usual manner. A phosphate citrate buffer at a pH of 5 was used. The alkaloids move to the cathode at this pH and a separation into

several fractions is accomplished in a 24-hour run using a potential of 200 volts and a current of 2 m.a., per inch width of paper. The papers were removed, air-dried and photographed under mercury arc ultraviolet light. A photograph of an electropherogram showing the patterns of opium from several different countries of origin are shown in Fig. 2.

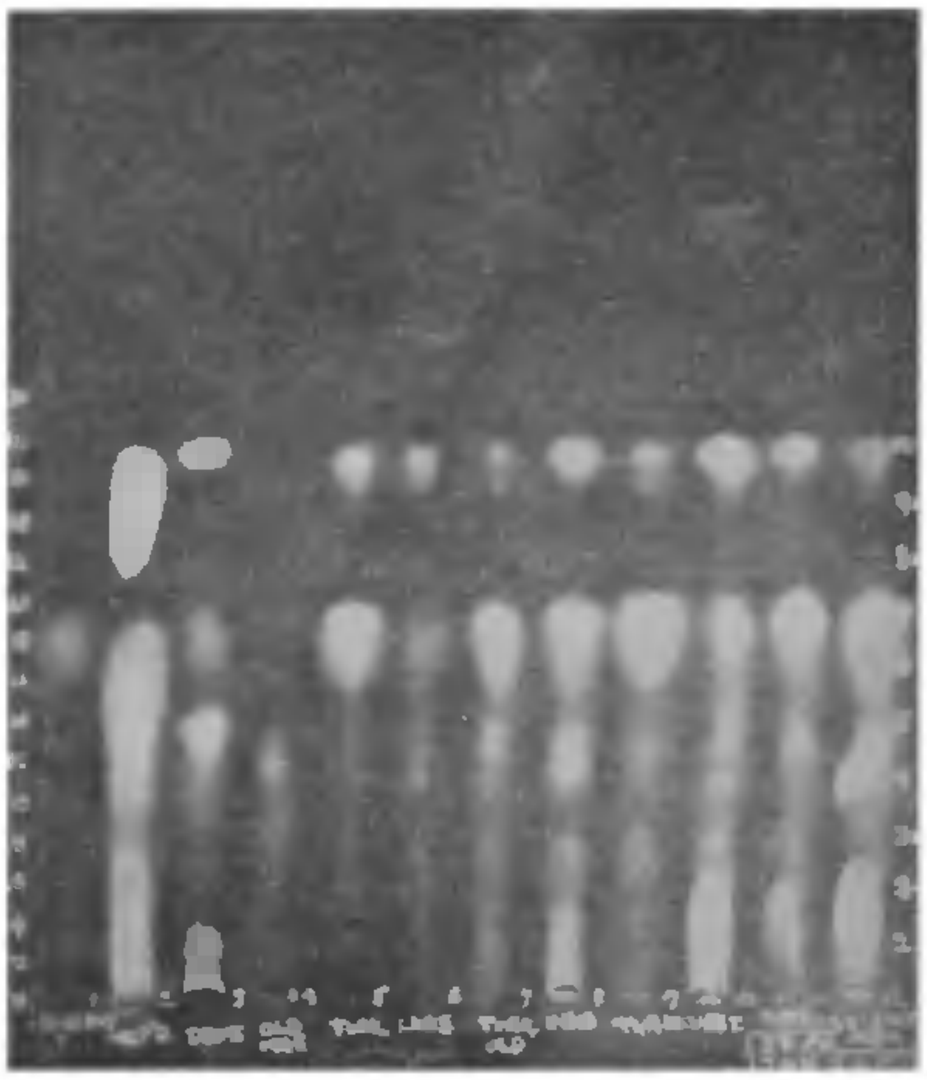

FIG. 2. Shows] electrophoretic patterns for opium extracts from opiums of different sources. The electropherograms were developed in a phosphate-citrate buffer at a pH of 5·0 as described in the text.

The data obtained suggests that this technique can be used for distinguishing opium samples of different countries of origin. Combined with the other established methods for determining origin such as the microscopic test, porphyroxine-meconidine value, codeine percentage and ash analysis, this technique of electrophoresis will permit positive origin identification of different samples of opium.

The toxicologist is required to detect the presence or absence of alkaloids and related basic drugs in viscera. The Stas-Otto process involves many steps and the final extract contains large amount of normal tissue extractives which interfere with the chemical and physical tests used in the identification of these drugs. A direct extraction of the tissue with a solvent like ethyl ether followed by the purification of ether extract using the technique of paper electrophoresis have many advantages. By this method, the suspected component can be separated from many normal extractives. The use of sensitive spotting agents makes for increased sensitivity in detecting or ruling out basic drugs. A quantitative estimation of concentration may be made by comparison of the spots with known standards. The compounds may be recovered in relatively pure state by proper elution and subsequent extraction by immiscible solvent.

After the run of electrophoresis, the dry paper strip is first examined in the dark with the ultraviolet light. Those basic drugs that are strongly fluorescent such as quinine, quinacrine and others are readily located in concentrations of less than 1 microgram. The fluorescence of these drugs is different from those of the normal tissue extractives. Compounds that strongly absorb ultraviolet light will be seen as dark areas on the paper. Strychnine and many other antihistaminic drugs can be located on the paper when present in concentrations of less than 10 micrograms. After marking the areas of fluorescence or absorption, the paper is sprayed with iodoplatinic acid. This reagent reacts with basic compounds to form blue or black areas. As little as 10 micrograms of most basic drugs can be detected with this reagent. For the detection of compounds such as adrenaline, amphetamine, methamphetamine and others, dinitrofluorobenzene is used as a spotting reagent. After first spraying the paper with iodoplatinic acid, any dark areas that appear are cut out and saved for elution. The remainder of the paper is sprayed with a solution of 2% sodium carbonate. This decolorises the brown iodoplatinic reagent and furnishes the necessary alkalinity for the reaction of primary amine with dinitrofluorobenzene. Deep yellow areas appear that remain coloured after dipping the dry paper in dilute hydrochloric acid, if aliphatic amines are present.

Fresh drug-free samples of tissues, especially, liver when carried through the above procedure, will contain small amounts of basic compounds. These compounds appear, when present, as fast moving components. They can be located as a dark spot in ultraviolet light and will form a black spot after spraying with iodoplatinic acid reagent. Another compound will appear after spraying with dinitrofluorobenzene indicating the aliphatic amine. The latter compounds are present in large amounts after putrefaction and will be in such high concentration as to give dark compounds with iodoplatinic acid.

By the employment of this technique, it will be possible to differentiate between the extraneous basic drugs, and the amine that might be

produced by putrefaction. Electrophoresis is, therefore, a valuable tool in the hands of toxicologists for giving a definite opinion as to the presence or absence of a basic drug or poison in viscera, particularly, when putrefaction has already taken place.

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HIGH RESOLUTION RAMAN SPECTRA OF C_2H_4 AND C_2D_4

THE unique importance of the molecular structure of ethylene for valence theory has made this molecule a favourite for spectroscopic studies at high resolution. Early studies of the pure rotational Raman spectrum of ethylene were interpreted in terms of a symmetric and near-symmetric top molecule. These studies have led to the much quoted parameters r (C = C) = 1·353 A, r (C-H) = 1·071 A, and ∠ (H-C-H = 120°, for the structure of the ethylene molecule.

Dowling and Stoicheff have given the results of a detailed analysis of the pure Rotational Raman Spectra of C_2H_4 and C_2D_4 under high resolution [*Canad. J. Phys.*, 37 (6), 703-21]. The spectra were photographed in the second order of a 21 ft. grating. The resolution achieved was high enough to warrant analysis based on the non-rigid asymmetric top. Several lines were identified as single transitions and their analysis has led to an accurate evaluation of the rotational constants for the ground states. The structural parameters of ethylene obtained from these constants are given below, compared with their values obtained by infra-red spectroscopy and electron diffraction method.

	r_0 (C=C)	r_0 (C–H)	∠H-C-H
Raman ..	1·339 ±0·002 Å	1·086 ±0·003 Å	117° 34′ ± 20′
Infra-red ..	1·337 ± ·003	1·086 ±0·003	117° 22′ ± 1°
Electron diffraction	1·334 ±0·003	1·085 ±0·005	116° ± 1°

NATIONAL PHYSICAL LABORATORY (ENGLAND)—ANNUAL REPORT FOR 1958*

THE Report outlines the recent reorganization at the NPL and the new trends in its research work. The replacement of three of the old Divisions by the new Divisions of Applied Physics, Basic Physics and Standards has enabled the Laboratory to plan research into new fields. The new Basic Physics Division is equipping itself to begin work mainly devoted to the relation between the macroscopic properties of materials and their structure on a molecular scale. In the Applied Physics Division, work has begun on radiocarbon dating and a national centre for neutron source standardisation is being formed. A new item in the Standards Division is the accurate determination of the gyromagnetic ratio of the proton which will open the way to precise measurement of strong magnetic fields and hence to the accurate determination of a number of important fundamental constants.

Other new aspects of the programme include the increasing attention of Aerodynamics Division to the behaviour of gases at the high temperatures associated with high speed flight and to the development by the Control Mechanisms and Electronics Divisions of new components for very high-speed computers. The Light Division has made progress with some interesting new ideas in the application of gratings to linear and angular measurement and to the control of machine tools. The Metallurgy Division has been concentrating particularly on fine structure of metals, movements of dislocations and their relation to physical and mechanical properties. A group is being set up in the Mathematics Division to consider the theoretical aspects of experimental work being carried out in other Divisions.

* Published for D.S.I.R. by H.M.S.O., price 8*s*.