artefacts. Given adequate control, there is no doubt a good degree of reproducibility; but owing to differing interactions between various components of different mixtures, the patterns observed can have no direct correlation with the complexities of the mixture.

Since this work was completed, Swingle and Tiselius[8] have also expressed the opinion that the patterns observed under these conditions are artefacts dependent on the general properties of proteins in contact with paper; but owing to the fact that our observations may be of interest in further work on protein separation, we have felt it desirable that they should be placed on record.

D. A. Hall
F. Wewalka

Department of Medicine,
University of Leeds.

[1] Tiselius, A., and Shepherd, C., Disc. Farad. Soc., No. 7, 275 (1949).
[2] Sober, H. A., Kegeles, G., and Gutter, F. J., *Science*, **110**, 564 (1949).
[3] Franklin, A. E., and Quastel, J. H., *Science*, **110**, 447 (1949).
[4] Franklin, A. E., and Quastel, J. H., *Proc. Soc. Exp. Biol. and Med.*, **74**, 803 (1950).
[5] Cremer, H. D., and Tiselius, A., *Biochem. Z.*, **320**, 273 (1950).
[6] Durrum, E. L., *J. Amer. Chem. Soc.*, **72**, 2943 (1950).
[7] Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, **111**, 605 (1950).
[8] Swingle, S. M., and Tiselius, A., *Biochem. J.*, **48**, 171 (1951).

A technique for the two-dimensional chromatography of blood plasmas or protein mixtures on filter paper has been described by Franklin and Quastel[1] which differs in certain important respects from the well-known methods adopted in amino-acid paper chromatography. It was found that the additions of small quantities of surface active agents such as the 'Tweens' or 'Spans' seemed to facilitate the separation of plasma constituents and extend the protein 'pattern'. The technique adopted at present is to add 'Tween 85' or 'Tween 81' to the plasma and use hæmin as the protein marker. A mixture of an alcoholic solution of benzidine and hydrogen peroxide, made acid with acetic acid, is used for detecting the hæmin. Solutions of sucrose and of sodium potassium tartrate have been selected, after many experiments, as the most suitable developing solvents in the first and second dimensions respectively. The former solution appears to facilitate movement of certain protein hæmin complexes, as well as uncombined hæmin, in the first dimension; the latter solution allows movement of certain protein complexes in the second dimension while preventing movement of uncombined hæmin. There must not be overloading of the paper with protein; usually 0·02 ml. plasma is applied. The technique has been used in the study of blood plasma changes that occur consistently after heparin administration and after single high-fat meals[2] and in investigations of multiple sclerosis[3]. It has also been used profitably in an investigation of the combination between thyroxine and plasma constituents[4], in a study of the breakdown of casein by rennin and of the separation of rennin from crude rennet preparations[5], and in the demonstration of non-specific agglutinins for Brucella in bovine sera[11].

Drs. Hall and Wewalka point out that the upward flow of the plasma proteins from the original spot on the paper consists of a gradual elution by the ascending solvent and conclude "that the relative positions of the components of a mixture are dependent on certain factors not necessarily functions of its complexity". They also state, without giving the experimental evidence, that at no stage in the process is there a linear distribution of the various components, albumin and globulin bands appearing in the final electrophoresis patterns of all the areas of the spot (no surface-active agents were used). Thus, they intimate that no separation of protein constituents of the plasma takes place in the first-dimensional run. This conclusion leads them to imply that the fractionations seen in the second dimension are due simply to the mechanical or physical factors operating in this technique, and do not reflect any separations of a chemical nature. It is further claimed that the action of surface-active agents is only to prevent the coalescence of fractions which is observed in the second-dimensional run when no surface-active agent is present.

If these observations and conclusions are correct, it follows that the protein patterns of various plasmas should offer no great dissimilarities, since the patterns are allegedly not dependent on the chemical complexity of the plasmas but on the physical factors operating in the runs in the two dimensions.

Much experience has shown that the protein patterns obtained using our technique with normal plasmas are very similar to each other and may be reproduced with considerable regularity. On the other hand, the patterns obtained with many pathological plasmas differ very greatly from those given by the normal. These may also be duplicated with regularity in the same patient, so long as the clinical condition of the patient does not alter. There is now ample evidence which, it is hoped, will be published shortly, to show that the abnormal patterns observed in certain diseases revert to normal when the disease is successfully treated. Photographs of typical paper chromatograms of (*a*) normal plasma, (*b*) plasma of a patient suffering from cancer of the

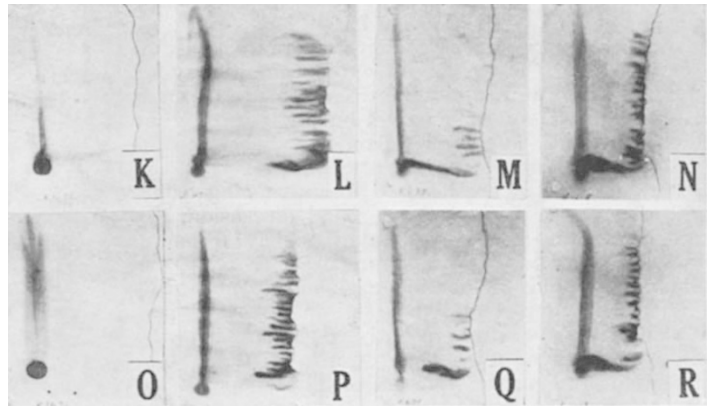

Fig. 1. *K*, Hæmin control (0·02 ml. of 0·3 per cent hæmin in 0·5 ml. of distilled water plus 0·02 ml. of 'Tween 81'); *L*, normal plasma (0·02 ml. of 0·3 per cent hæmin and 0·02 ml. of 'Tween 81' added per 0·5 ml. of plasma); *M*, plasma of a patient with cancer of the stomach (0·02 ml. of 0·3 per cent hæmin and 0·02 ml. of 'Tween 81' added per 0·5 ml. of plasma); *N*, plasma of a patient with lupus erythematosus (0·02 ml. of 0·3 per cent hæmin and 0·02 ml. of 'Tween 81' added per 0·5 ml. of plasma); *O*, same as *K* but with 0·01 ml. of 'Tween 85' instead of 'Tween 81' added per 0·5 ml. of plasma; *P*, same as *L* but with 0·01 ml. of 'Tween 85' instead of 'Tween 81' added per 0·5 ml. of plasma; *Q*, same as *M* but with 0·01 ml. of 'Tween 85' instead of 'Tween 81' added per 0·5 ml. of plasma; *R*, same as *N* but with 0·01 ml. of 'Tween 85' instead of 'Tween 81' added per 0·5 ml. of plasma

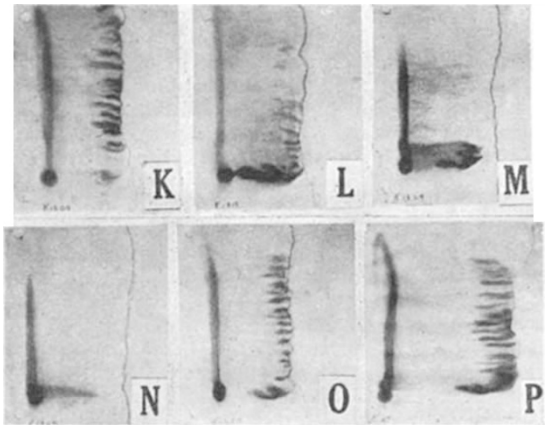

Fig. 2. *K*, Cohn fraction V (95 per cent albumin and 4 per cent α-globulin), 0·02 ml. of 0·3 per cent hæmin and 0·02 ml. of 'Tween 81' added to 0·5 ml. of a 6·5 per cent solution; *L*, Cohn fraction IV-4 (16 per cent albumin, 46 per cent α-globulin and 38 per cent β-globulin); experimental conditions as in *K*; *M*, Cohn fraction II (3 per cent β-globulin and 97 per cent γ-globulin); experimental conditions as in *K*; *N*, Cohn fraction I (7 per cent albumin, 8 per cent α-globulin, 15 per cent β-globulin, 9 per cent γ-globulin and 61 per cent fibrinogen); experimental conditions as in *K*; *O*, reconstituted plasma (3 per cent Cohn fraction V, 1·3 per cent Cohn fraction IV-4, 0·5 per cent Cohn fraction II, and 0·6 per cent Cohn fraction I in 0·85 per cent saline); experimental conditions as in *K*; *P*, normal human plasma; experimental conditions as in *K*

stomach, (*c*) plasma of a patient suffering from lupus erythematosus are shown in Fig. 1 (see also ref. 6, where further results are presented). These facts, pointing to extensive differences between the patterns of normal and pathological plasmas, make it obvious that the nature of the two-dimensional pattern must be a function of the chemical composition of the plasma.

That the physical factors operating in this form of paper chromatography help to determine the final pattern need scarcely be said, and this, it seems to us, is inevitable. But this fact does not justify the conclusion that the pattern does not reflect the chemical composition of the plasma or protein mixture. Moreover, no statement has been made in our publications that the fractions seen in the paper chromatograms of plasmas represent separate protein species.

Definite separations of proteins are practicable by this technique. For example, cytochrome *c* may be separated from serum albumin in a mixture of these substances[6]. The former moves but little in the first dimension and is easily taken into the second dimension, whereas the latter moves freely in the first dimension, to the head of the advancing front, and also moves easily in the second dimension. It can be shown (see Fig. 2) that widely different patterns occur with purified preparations of serum albumin, α- and β-globulins, γ-globulin and fibrinogen using our technique. Papastamatis and Wilkinson[7] have also recently demonstrated the different rates of movement of insulin, fibrinogen, γ-globulin and serum albumin, on filter paper using salt solutions as developing solvents.

The paper chromatogram of a mixture of serum albumin, serum globulins and fibrinogen in the proportions in which they are present in plasma gives a pattern very similar to that obtained with a normal plasma (see Fig. 2). This is a further indication that the chromatogram of a protein mixture is dependent on the chemical composition of its constituents.

Hæmin alone, in the absence of added proteins, under our experimental conditions gives but little movement in the second dimension, and this is negligible compared with the movement of the protein–hæmin complexes (see Fig. 1). Drs. Hall and Wewalka point out that movement of hæmin, in the absence of added proteins and in the presence of 'Tweens', does take place in the second dimension; but they use a fructose solution as developing agent in *both* dimensions. Naturally, in such circumstances, a movement in the second dimension will take place; but this is quite irrelevant since they do not use our developing solvent, which suppresses the movement in the second dimension.

Furthermore, Drs. Hall and Wewalka assert that the action of the surface-active agents is due to a 'wetting-out' phenomenon whereby the fractions obtained in the first direction are not allowed to coalesce in the second. Thus, the appearance of greater fractionation is observed. But this assertion does not take into account the fact that, in the absence of the surface-active agent, serum albumin will ascend wholly to the head of the paper, there to be separated into a few fractions in the second dimension[8], whereas in the presence of suitable quantities of a 'Tween' the rate of movement of the protein is depressed and a considerably lengthened spot is obtained in the first dimension, giving rise to an increased number of fractions. With a serum globulin, the presence of the 'Tween' may suppress all movement in the first dimension (see Fig. 2).

Combinations of detergent and enzyme proteins have been described[8] and it is known from the work of Putnam and Neurath[9] that albumins combine with surface-active agents such as the detergents to form complexes of various mole ratios. It is therefore likely that the elongation of the protein spot in the first dimension may be partly due to the different rates of solution of the surface-active agent – protein complexes and partly due to the varying affinities of these complexes for the cellulose of the paper. The increase, therefore, in the number of fractions is probably largely due to the formation of these complexes. There can be little doubt that complex formation between globulins and surface-active agents, such as 'Tweens', also occurs and that these complexes move with great difficulty in the first dimension under our experimental conditions. Use of other surface-active agents may provide for more effective separations of albumins and globulins than we have been able to obtain so far.

Further experiments[6] have shown that the admixture of γ-globulin to human plasma brings about a marked alteration of the pattern, a heavy basal fraction appearing when 'Tween' is used. The heavy fractions at the bottom of the pattern seem to be typical of the chromatograms obtained in plasmas of

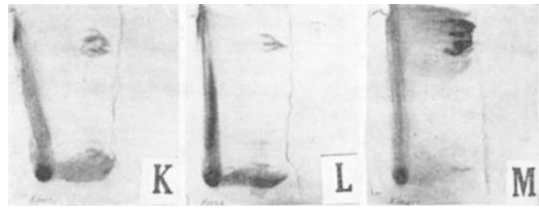

Fig. 3. Cobra venom: *K*, supplied by Haffkine Institute, Bombay; and *L*, supplied by Hynson, Westcott and Dunning, Inc., Baltimore, Md.; *M*, *Agkistrodon piscivorus* venom supplied by Ross Allen's Reptile Institute, Silver Springs, Florida

pathological cases where the globulin/albumin ratio has been increased. It has already been pointed out[1] that the chromatogram of the plasma of a horse immunized against diphtheria toxin (the plasma presumably having an increased globulin content) differs from that of normal horse plasma by the possession of extra fractions at the bottom.

The fact that protein mixtures of different chemical composition yield different patterns is also illustrated by the photographs given in Fig. 3. These show chromatograms of two different samples of cobra venom and of a sample of *Agkistrodon piscivorus* venom, the same quantity (0·8 mgm.) being taken, and no surface-active agents being present. It will be observed that the chromatograms of the two cobra venoms are very similar to each other but differ from that of the other venom.

It is difficult to reconcile two statements in Drs. Hall and Wewalka's communication, namely, "The separation of proteins of quite dissimilar nature by this method, however, is, of course, perfectly practicable", and "the patterns observed in complex mixtures such as serum are so dependent on the conditions of the experiment as to be pure artefacts". We readily agree that all patterns are dependent on the conditions of the experiment; but since separations of proteins admittedly do occur by this technique, the patterns cannot be regarded as solely dependent on the physical conditions and therefore as pure artefacts.

With reference to the suggestion of Swingle and Tiselius[10] that the chromatograms may be "artefacts", we are informed by Dr. Swingle[12] that the evidence referred to in their paper was rather circumstantial and did not seem to justify publication without additional experiments.

Further investigation, especially of a quantitative nature, is needed to decide the chemical nature of the fractions obtained in the second dimension, and such work is now in progress. But ignorance of this fact, at present, need not deter further development of the technique, which is obviously in an exploratory stage. It is possible that it will provide an inexpensive means of clinical aid both in diagnosis and in following treatment.

A. E. Franklin
J. H. Quastel
S. F. Van Straten

Research Institute,
Montreal General Hospital,
Montreal.
July 17.

[1] Franklin, A. E., and Quastel, J. H., *Proc. Soc. Exp. Biol. Med.*, **74**, 803 (1950).
[2] Swank, R. L., Franklin, A. E., and Quastel, J. H., *Proc. Soc. Exp. Biol. Med.*, **75**, 850 (1950).
[3] Swank, R. L., Franklin, A. E., and Quastel, J. H., *Proc. Soc. Exp. Biol. Med.*, **76**, 183 (1950).
[4] Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, **111**, 605 (1950).
[5] McKerns, K. W., *Canad. J. Med. Sci.*, **29**, 59 (1951).
[6] Franklin, A. E., Quastel, J. H., and Van Straten, S. F., *Proc. Soc. Exp. Biol. Med.* (in the press).
[7] Papastamatis, S. C., and Wilkinson, J. F., *Nature*, **167**, 724 (1951).
[8] Hunter, A., and Downs, C. E., *J. Biol. Chem.*, **173**, 31 (1947). See also Mayer, R. L., and Eisman, P. C., *Proc. Soc. Exp. Biol. Med.*, **77**, 452 (1951).
[9] Putnam, F. W., and Neurath, H., *J. Amer. Chem. Soc.*, **66**, 1992 (1944); *J. Biol. Chem.*, **159**, 195 (1945); **160**, 397 (1945).
[10] Swingle, S. M., and Tiselius, A., *Biochem. J.*, **48**, 171 (1951).
[11] Hess, W. R., and Roepke, M. H., *Proc. Soc. Exp. Biol. Med.*, **77**, 469 (1951).
[12] Swingle, S. M., private communication.

ANNUAL EXHIBITION OF THE ROYAL PHOTOGRAPHIC SOCIETY, 1951

THE ninety-sixth annual exhibition of the Royal Photographic Society was opened in London on September 13 for one month and will also be shown at the City Art Gallery, Aberdeen, during November 3–24. The exhibition is divided into five groups: pictorial, record, Nature, medical and scientific photography, with a total of 908 exhibits.

The scientific entries constitute the smallest individual group; but medical and surgical entries are well represented. There is a collection of some nine full-sized radiographs in this latter section, one of which is very effectively shown as a two-colour radiograph by D. Stevenson Clark and entitled "Bas relief colour radiograph of right pneumothorax". This was prepared from the original radiograph by contact-printing a positive to give a magenta image and registering this with a solarized negative coloured cyan. One of the other monochrome radiographs, by R. M. Leman, was given a distinct relief effect presumably by the use of a slightly out of register negative mask, which resulted in a noteworthy emphasis of the arteries shown in this particular exhibit. Among the medical prints is one from Manchester Royal Infirmary, in which the arteries of a heart are well revealed by a radiograph made after injection of lead phosphate. Included in the several examples of ophthalmic photography are two, one by P. N. Cardew and the other by Miss A. E. Milne, in which an annular electronic flash tube was used to illuminate the eye. The reflexion of the tube itself by the outer surface of the cornea indicates whether any imperfections exist on this surface, these being revealed by distortion in the shape of the annulus. Two exhibits by D. M. Kermack showing stages in the dissection of a marine worm provide an illustration of how revealing and orderly a careful example of this type of work can be. A series of photomicrographs by G. C. Lenney on cell division in a mouse tumour must have caught the eye of any who have visited the Festival of Britain Science Exhibition, where a large section is devoted to cell division: in these photographs both normal double-cell division and abnormal multi-cellular division showing stickiness of the chromosomes are well shown.

The most interesting exhibits in the science section are those submitted by the National Physical Laboratory. In one of these there is an example of the use of the Linnick interference microscope, in which the relief effect shown on the surface of a photographic negative of a three-bar resolution test object is examined. Horizontally, the magnification is × 2,100; but by virtue of the optical interference shadows an effective vertical magnification of × 166,000 is achieved. The actual relief effect in the gelatin of the image under examination is seen to be almost one micron. The Laboratory also shows some examples of the *Schlieren* patterns produced by high-velocity air flow. Thus the air flow in the neighbourhood of a supersonic jet is shown, as are the pneumatic disturbances produced around an aerofoil at high subsonic and also supersonic speeds. The shock wave produced in the former case and the 'bow wave' in the latter are clearly indicated. By using the properties of polarized light, the degree of air compression in these waves can be assessed by