eterotermi. Il tenore enzimatico degli eritrociti dei Mammiferi è inferiore (a parità di volume protoplasmatico) a quello di tutti gli altri Vertebrati studiati.

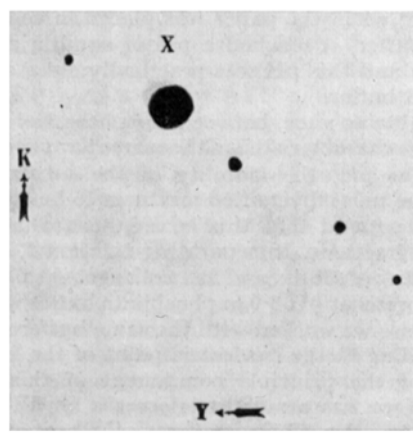
Dall'insieme delle ricerche è risultata l'influenza del nucleo e della termoregolazione (dedotta dalle condizione eteroterme e omeoterme degli organismi studiati) sul tenore in dipeptidasi degli eritrociti nucleati e anucleati dei Vertebrati.

Some Remarks on Two-Dimensional Paper Electrophoresis[1]

Paper electrophoresis has been making rapid development during recent times both on account of the simplicity of carrying out the method and the low cost of the equipment required. But to be sure that it is really paper electrophoresis and not just chromatography with applied voltage, the process should be carried out under conditions which ensure having a movement of the substance which depends essentially on the electric field applied, and exclude other factors which might interfer with the final result. From this point of view, flat electrophoresis presents undoubted advantages over that carried out on an inclined surface, even with continuous evaporation of the solvent according to MACHEBOEUF and his associates[2] (electrorheophoresis).

In any case, paper electrophoresis effectively opens up a large field of inquiry on account of the various opportunities for successful combination with chromatography[3], also for effecting a continuous separation[4] of the different substances and for carrying out two-dimensional electrophoresis[5], that is, twice successively in directions at right angles to each other and under different electrical conditions.

Our working conditions[6] for the study of two-dimensional paper electrophoresis have been the same as those described by KUNKEL and TISELIUS[7], with the exception of one particular: the two opposite sides of the square sheet of paper did not dip into buffer, but were electrically connected there with small siphons of the same paper (four at each side) not larger than 15 mm. This arrangement, even though it forced us to work with a lower current intensity, operated in such a way that the movement of the substances was independent of their position on the paper and due exclusively to the forces of the electric field. In fact, also in electrophoresis in a single direction, we have noticed many times that when the paper strip was dipping at the two extremities for its entire width into buffer contained in the vessels, there was—even without the current—a movement of the substances due to the strong influx of electrolyte. This movement was negligible in the central part of the strip and increased little by little gradually as it approached the buffer in the vessels. These movements had a really noticeable influence on the results of the electrophoresis, particularly for amino acids and the more diffusible ions. When, in order to have a larger path available, a starting point was selected near one of the ends of the strip, it happened sometimes that, at first, the substances migrated towards the nearest vessel (pole with the same charge) before starting their normal route towards the opposite pole; other conditions being equal, the path taken by the substances differed according to the initial point. Even when the starting point was chosen in the central part of the strip, during the electrophoresis with the movement due to the electric field, the substances fell under the influence of the attraction exerted by the buffer. Although it was possible to determine this influence exactly at each point, both without the application of the current and when the strip was inserted in the circuit (and in this case such influence adds up algebraically to the value of the electroendosmotic flow), we preferred to try to eliminate this interference. The use of narrow siphons (one each seven centimetres of the width of the strip) eliminates, as we already said, this difficulty.



Two-dimensional paper electrophoresis of human plasma fraction I (according to COHN *et al.*). *X* = origin. *K* = direction in the first phase. Potential, 300 V. Duration, 360 min. Phosphate buffer, pH 8·0, ionic strength 0·01. *Y* = direction in the second phase. Potential, 450 V. Duration, 600 min. Phosphate buffer, pH 5·7, ionic strength 0·1. – From the left descending to the right: gamma-globulins, fibrinogen, beta- and alpha-globulins, albumin.

In our work we have always carried out two-dimensional electrophoresis on the horizontal plane and we have checked that the path taken is due almost exclusively to the migration of the ions under the difference of potential and to the electroendosmotic flow (the effects of this latter are proportionately reduced if MUNKTELL paper 20/150 is used). Working in the two phases of the process, with the same buffer (same pH and same ionic strength) the ratio between the speed of the individual components remains constant with variations of the difference of the potential applied and, at the end of the second phase, the various substances are found along a straight line, the position of which on the plane depends solely on the characteristics and duration of the passage of the current. Each individual component is found at the extremity of the hypotenuse of a right angled triangle, the other sides of which represent the path taken by the substance in the two different phases of the process.

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Two-dimensional electrophoresis is particularly useful for discovering whether or not the tail ends or the spreading out of the spots are due to extraneous electric factors.

The use of the small siphons described before also enables the pH of the buffer to be changed between one phase and another of the electrophoresis, without drying the sheet and without causing the substance to move; even simple drying in the air can cause alteration in the proteins. Usually we preferred not to change the salts of the buffer, since (as for free electrophoresis) this can cause alterations of the mobility, independent of the variation in pH.

Usually we carried out the first phase of the electrophoresis with a buffer having a weak ionic strength, and the second phase with a buffer of higher ionic strength. The salts of the buffer were the same but in different proportions and, therefore, the pH was different. In the interval between the two phases, the sheet of paper (always kept between the two glass plates) was raised so that the siphons no longer dipped into buffer, and then was lowered again (turned 90 degrees) only when the buffer had been changed.

The sheets of paper that we used (MUNKTELL paper 20/150 in squares of 30·5 cm each side) retained under our experimental conditions about 35 ml of solutions of electrolyte; when the paper was placed in contact with the new buffer, it reached a pH of equilibrium within 10–15 min and this pH was practically equal to that of the second buffer.

This ability to vary, between one phase and the other, not only the characteristics of the current applied but also, through the pH, the mobility of the substances, has enabled the most diversified mixtures to be studied.

We have found that this is very useful in studying plasmatic fractions. In separating fraction I, according to method 6 of COHN and his colleagues[1], after a first electrophoresis at pH 8·0 in phosphate buffer, during the second phase we worked with the same buffer with a pH corresponding to the isoelectric point of the fibrinogen, that is, of the principle component of this fraction. Even if there was a mobility decrease for all the other components, the fibrinogen remained at the point reached at the end of the first phase, and if the second period of the electrophoresis lasted a sufficiently long time, it was possible to obtain a clear cut separation, which was very useful from a quantitative point of view.

One should bear in mind, however, that the isoelectric points noted are always slightly higher than those found in free electrophoresis[2], and perhaps this is due to the influence of the electroendosmosis. In any case, we have always preferred to work under the conditions described rather than try to overcome this influence, raising the level of the buffer in the cathode compartment. For example, an isoelectric point at pH 5·68–5·73 was observed for fibrinogen in phosphate buffer of 0·1 ionic strength in comparison with that of 5·53 checked in free electrophoresis.

G. DICASTRO

«Medital» Laboratories Research Division, Rome, July 28, 1953.

Riassunto

Per una buona esecuzione dell'elettroforesi su carta è necessario lavorare con alcune precauzioni, che rendono i risultati indipendenti dalla posizione iniziale delle sostanze. In tal modo è anche possibile eseguire l'elettroforesi bidimensionalmente, con cambiamento del pH e della forza ionica del tampone tra una fase e l'altra, modificando così le velocità di migrazione.

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Phosphorylation Mechanisms in Cloudy Swelling

Previous papers have shown that the purest form of experimental cloudy swelling is obtained when animals have been intoxicated with bacterial toxins[1]. The mild morphological changes which occur in such a cellular metamorphosis are constantly accompanied by chemical and enzymatic alterations, e.g. the protein-N is increased[2], DNA content is lowered in cells of the renal cortical tubules[3], the alkaline phosphatase distribution in the kidney is modified[4] and its activity markedly decreased[5]. In renal cells oxygen uptake and fatty acid oxidase activity are depressed by cloudy swelling[6]. Moreover, it has been observed that cyclophorase preparations from kidney, brain, heart and skeletal muscle of diphtheria intoxicated guinea-pigs are inhibited in their capacity to catalyze the processes of the tricarboxylic acid cycle in the later stages of the intoxication[7].

It has been known since long that mitochondrial changes occur in cloudy swelling, and recently ZOLLINGER[8] added new and interesting data about it. Furthermore, it has been pointed out that heart-mitochondria, caused to swell by changing the electrolyte concentration of the medium, show a lowered cyclophorase activity[9] and a decreased P:O ratio[10].

Recent research has shown that in diphtheria intoxicated rabbits, whose organs are notoriously affected by cloudy swelling, the glucose charge does not produce the lowering of inorganic phosphate concentration in blood so much as in the normal control[11]. Hypophosphataemia occurring in normal animals after glucose injection is due to an increased phosphate uptake in tissues, i.e. it depends upon the phosphorylation of the introduced glucose.

It therefore appeared reasonable to assume that in the diphtheria intoxicated animals the functional activity of the phosphorylating systems could be decreased.

In order to clarify the mechanisms involved, the hexokinase activity and the acid-soluble phosphorus fractions have been determined in guinea-pig tissues, both normal and affected by cloudy swelling. The phosphorus fractions have been studied, both in the controls and in the experimental animals, either fasting or after glucose charge.

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