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.

[1] E. J. COHN et al., J. Amer. Chem. Soc. *68*, 459 (1946).
[2] V. SCHWARZ, Nature (London) *167*, 404 (1951).

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.

[1] A. FONNESU, Boll. Soc. ital. Biol. sper. *28*, 482 (1952); Arch. Sci. biol. (in press). – A. FONNESU and C. SEVERI, Brit. J. exp. Path. *34*, 341 (1953); Riv. Biol. *44*, 381 (1952).
[2] G. POPJÁK, J. Path. Bact. *60*, 75 (1948).
[3] A. FONNESU and C. SEVERI, Brit. J. exp. Path. *34*, 341 (1953).
[4] A. FONNESU and C. SEVERI, Riv. Biol. *44*, 381 (1952).
[5] C. SEVERI, Boll. Soc. ital. Biol. sper. *28*, 1925 (1952).
[6] A. FONNESU, Boll. Soc. ital. Biol. sper. *28*, 482 (1952); Arch. Sci. biol. (in press).
[7] O. HOFFMANN-OSTENHOF, O. F. SCHWARZ, W. ZISCHKA, and H. EIBL, II[e] Congr. int. Biochimie, Paris; Communications 35 (1952).
[8] H. U. ZOLLINGER, Exper. *4*, 312 (1948); *6*, 14 (1950); Rev. Hématol. *5*, 696 (1950).
[9] J. W. HARMAN, Amer. J. Pathol. *26*, 687 (1950); Expt. Cell. Res. *1*, 394 (1950).
[10] J. W. HARMAN and M. FEIGELSON, Expt. Cell. Res. *3*, 509 (1952).
[11] A. FONNESU and C. SEVERI, Boll. Soc. ital. Biol. sper. *28*, 1923 (1952).

Cloudy swelling in guinea-pig tissues was produced experimentally by subcutaneous injection of diphtheria toxin (1 M.L.D./250 g of body weight). Liver, kidney, heart and skeletal muscle were taken 24 h after the toxin injection.

Hexokinase activity was estimated in tissue homogenates according to LONG[1].

Fractionation of acid-soluble phosphorus was carried out as described by PINCHOT and BLOOM[2] and phosphorus concentration in the fractions determined by the method of FISKE and SUBBAROW[3].

Table I

Hexokinase activity of tissues of guinea-pig injected with diphtheria toxin compared with normal controls

Tissue	$-Q_{glucose}$[1] Normal guinea-pig	Diphtheria intoxicated guinea-pig
	[2]	[2]
Kidney (whole) . .	*10,0* 9,1–10,6 (3)	*10,1* 9,6–10,6 (3)
Heart	*8,6* 6,9–9,6 (3)	*8,4* 6,3–9,8 (3)
Skeletal muscle . .	*5,4* 4,4–6,6 (3)	*5,3* 3,9–6,2 (3)
Liver	*1,6* 1,4–1,9 (3)	*1,8* 1,4–2,0 (3)

[1] $-Q_{glucose}$ = µl glucose disappearing/mg dry weight/h, where 180 µg glucose = 22,4 µl.

[2] Mean, range and number of observations (in parentheses).

The glucose charge was performed with intracardiac injection of 0·2 g of glucose/100 g of body weight. Tissues employed for fractionation of acid-soluble phosphorus were removed from animals under $MgSO_4$ anaesthesia; when animals were given glucose, anaesthesia began at a time corresponding to the lowest level of inorganic phosphate concentration in the blood (15 min).

[1] C. LONG, Biochem. J. *50*, 407 (1951).

[2] G. B. PINCHOT and W. L. BLOOM, J. biol. Chem. *184*, 9 (1950).

[3] C. H. FISKE and Y. SUBBAROW, J. biol. Chem. *66*, 375 (1925).

The results of the hexokinase activity measurements are summarized in Table I; the data show clearly that the enzymatic activity is unchanged in tissues affected by cloudy swelling, as compared with controls.

The results of the phosphorus determinations in normal animals and in animals injected with diphtheria toxin, both examined fasting and after glucose charge, cannot be reported here extensively and are briefly summarized in Table II.

The following fractions have been considered: 180 min P, ester P, labile P (ATP, ADP), phosphocreatine P (PCP) and total high energy bond phosphorus (total ~P). All the fractions have been studied in liver, kidney, heart and skeletal muscle.

The mean values of the phosphorus determinations, carried out in tissues of fasting diphtheria intoxicated guinea-pigs, show, as compared with those of the relative controls, a statistically significant increase of inorganic P in liver and a significant decrease of phosphocreatine P, with a comparable rise of inorganic P, in muscle. A comparable fall of total ~P takes place since ATP is unchanged. It should be noted here that our findings for muscle are well in agreement with the data found by PINCHOT and BLOOM[1].

No significative change of the phosphorus-content in the fractions is induced in tissues of normal guinea-pigs by the injection of glucose. On the contrary, the glucose-charge in guinea-pigs treated with diphtheria toxin causes, in kidney and in muscle, a significant increase of inorganic P with a fall of ATP and of total ~P. No further lowering of phosphocreatine P, already decreased in the fasting intoxicated animals, is induced by glucose injection in diphtheria intoxicated guinea-pigs.

The evidence presented shows, therefore, that in cloudy swelling a marked fall in total high energy bond phosphorus takes place. This is particularly evident after glucose charge because the lowering involves both phosphocreatine and ATP.

Although a significant decrease of total ~P is observed only in certain tissues and chiefly in muscle, yet it appears probable that the phenomenon may also occur in other tissues, where the ~P content is too small to appreciate the change.

From the results referred in the present paper it seems quite reasonable to conclude that in tissues affected by cloudy swelling the formation of ~P is markedly reduced.

[1] G. B. PINCHOT and W. L. BLOOM, J. biol. Chem. *184*, 9 (1950).

Table II

Acid-soluble phosphorus fractions in organs of diphtheria intoxicated guinea-pigs either fasting or after glucose charge
Variations per cent from the control values[1]

Fraction	Diphtheria intoxicated guinea-pig											
	Fasting				After glucose charge							
	Liver	Kidney	Heart	Muscle	Liver		Kidney		Heart		Muscle	
	[2]	[2]	[2]	[2]	[3]	[4]	[3]	[4]	[3]	[4]	[3]	[4]
180 min P.	—	—	—	—	—	—	—	—	—	—	—	—
Ester P	—	—	—	—	—	—	—	—	—	—	—	—
Labile P (ATP, ADP).	—	—	—	—	—	—	—	−60%	—	—	−42%	−47%
PCP	—	—	—	−52%	—	—	—	—	—	—	−37%	—
Inorganic P	+34%	—	—	+27%	+59%	—	+20%	—	—	—	+57%	+24%
Total ~P.	—	—	—	−16%	—	—	—	−51%	—	—	−40%	−34%

[1] Only the changes statistically significant are reported (mean values).

[2] Relative to the normal fasting guinea-pig.

[3] Relative to the normal guinea-pig receiving glucose.

[4] Relative to the fasting diphtheria intoxicated guinea-pig.

Such a conclusion offers a satisfactory explanation of the disturbance observed in diphtheria intoxicated animals, i.e. the reduced hypophosphataemia after glucose charge in spite of the normal hexokinase activity.

Moreover, since the formation of ~P occurs chiefly in the mitochondria, it appears very probable that the metabolic alteration may be in relation to the known mitochondrial changes observed in cells in cloudy swelling.

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Institute of General Pathology, University of Perugia, Italy, February 25, 1953.

Riassunto

Negli organi in rigonfiamento torbido sperimentale da tossina difterica l'attività esocinasica è immodificata, mentre è rallentata la formazione di ~P. Ciò spiega perchè, negli animali intossicati con tossina difterica, il carico di glicoso determina ipofosfatemia meno netta. La diminuita formazione di ~P viene messa in rapporto con le alterazioni mitocondriali osservate nelle cellule in rigonfiamento torbido.

Essais d'inhibition de l'action inflammatoire cutanée du chloroforme chez le rat

L'application du chloroforme sur la peau du lapin provoque à l'endroit irrité l'apparition des colorants vitaux préalablement injectés par voie intraveineuse. AMBROSE et DE EDS ont donné au phénomène une expression quantitative fonction de sa période latente[1]. Dans une note antérieure[2], nous avons signalé l'influence de la cortisone, de l'A.C.T.H. et des salicylés sur le délai d'apparition des colorants au niveau de la peau irritée conformément à la technique originale de ces auteurs. Aujourd'hui, nous adapterons au rat le test d'AMBROSE et DE EDS et étudierons ses variations sous l'influence d'agents hormonaux ou pharmacodynamiques doués, selon la littérature, de propriétés antiinflammatoires. Nous rechercherons aussi chez cet animal l'influence de la surrénalectomie sur l'apparition du colorant au niveau des téguments irrités par le chloroforme.

Technique. Nous utilisons des rats adultes albinos, d'un poids moyen de 150 g provenant de deux élevages différents. Aucune différence entre les lots n'a été constatée.

1° La peau du dos est rasée la veille de l'expérience. Le lendemain, les rats reçoivent, par voie intrapéritonéale, 1 ml d'une solution de chlorazol blue sky, à 1% dans du sérum physiologique. Trois heures après, les animaux sont fixés sur le ventre. Un tampon de papier filtre de 2 cm² environ, imbibé de chloroforme et rapidement essoré, est appliqué fermement sur la peau durant 30 s. Un chronomètre est alors mis en marche et le temps minimum nécessaire pour qu'apparaissent les premières traces de coloration bleuâtre au niveau du derme est noté.

Deux tests sont pratiqués sur le même animal, de façon symétrique par rapport à la colonne vertébrale.

2° Les substances suivantes ont été administrées avant de pratiquer le test au chloroforme.

A.C.T.H.: 1 mg/100 g de poids du corps par voie intramusculaire deux fois par jour pendant 48 h.

Extrait cortical total: 0,25 ml de Cortine Organon (1 ml = 50 g de glande fraiche), une fois par jour, durant 48 h.

Cortisone: 1 mg/100 g de poids du corps, deux fois par jour, pendant 48 h, par voie intramusculaire.

Salicylate de soude: 300 mg/kg de poids du corps, deux fois par jour par voie intrapéritonéale, pendant 48 h.

Pour ces quatre produits, la dernière injection a lieu au moment de l'injection intrapéritonéale du colorant.

Phénergan: 5 mg par voie intramusculaire et 5 mg par voie souscutanée lors de l'injection du bleu.

3° La surrénalectomie a été pratiquée par voie dorsolombaire sous courte anesthésie à l'éther, la veille de l'administration du bleu. Certains animaux surrénalectomisés ont reçu de l'extrait cortical total à raison de 0,5 ml de Cortine Organon au moment de l'intervention. Chez d'autres qui ont reçu la même dose de Cortine au moment de la surrénalectomie, 300 mg/kg de salicylate de soude ont été injectés par voie intrapéritonéale au moment de l'administration du colorant vital.

Résultats. 1° Les tableaux I et II mentionnent, exprimés en secondes, les délais moyens d'apparition de la coloration bleuâtre au niveau du derme irrité.

2° La surrénalectomie entraîne une mortalité importante des animaux injectés de bleu, plus encore des animaux injectés simultanément de bleu et de salicylate de soude. La Cortine prolonge nettement la survie des animaux injectés.

[1] A. AMBROSE et F. DE EDS, J. Exp. Pharmacol. Therap. *90*, 359 (1947).

[2] H. VAN CAUWENBERGE et J. LECOMTE, Exp. *8*, 469 (1952).

Tableau I

Lots	Nombre d'essais	Délai moyen en secondes	σ de la moyenne*	θ coefficient de Bernoulli•
Témoins . . .	48	456	201	
A.C.T.H. . . .	68	253	84	0,99
Cortine	34	306	107	0,69
Cortisone . . .	36	340	179	0,46
Salicylate . . .	16	678	218	0,79
	16	>7200	—	—
Phénergan . .	27	1197	770	0,93

Tableau II

Lots	Opérés	Décès	Nombres d'essais	Délai moyen en secondes	σ de la moyenne*	θ coefficient de BERNOULLI •
Témoins	0	0	48	456	201	
Surrénalectomie	22	15	14	>7200	—	—
Surrénalectomie + Cortine	14	2	24	1296	422	1,81
Surrénalectomie + Cortine + Salicylate	12	6	12	1853	363	1,97

* σ: écart statistique: $\sqrt{\sum (x-\bar{x})^2/n}$. • θ: coefficient de probabilité: $X^1-\bar{X}/\sqrt{\sigma_1^2+\sigma_2^2}$.