PRO EXPERIMENTIS

The Interference of Phenols in the Fluorometric Determination of Nucleotides

LOWRY *et al.*[1] described a fluorometric method by which both oxidised and reduced phosphopyridine nucleotides, DPN, TPN, DPNH, and TPNH could be determined in animal tissues. An attempt was made to apply this method to plant tissue in view of the importance of a knowledge of such ratios in plants.

Homogenates of etiolated pea or lettuce seedlings were prepared in tris buffer and analysed by the method of LOWRY. No nucleotides could be detected. In recovery experiments where DPN was added to the homogenates, a loss of DPN of between 30 and 60% was observed. This loss was apparently not due to enzymic destruction since it was observed also when extracts of pea tissue were prepared in ice cold methanol and even after boiling such extracts. It appeared likely that some compounds in the extracts were interfering either with the development of fluorescence of the nucleotides during alkali treatment or were acting as quenching agents. Phenolic substances are widely present in plant tissue and are known to form oxidation products in strong base which might be responsible. A number of such compounds were therefore tested. The compounds were added to the DPN solution either before adding alkali and keeping at 38° C for 1 h or immediately before dilution for reading the intensity of fluorescence. Some of the results obtained are given in the Table. The recovery of DPN is calculated from the expected and observed fluorescence. An Eel fluorimeter having a primary filter transmitting at 365 mμ and a secondary filter with a transmission peak at 465 mμ was used. All tests were made in a range where the fluorescence of DPN and quinine sulphate is linear with concentration.

Effect of various substances on the fluorescence of DPN at a final concentration 1 μg/ml

Compound	Concentration	% Recovery of DPN if compound added	
		Before adding alkali	Before dilution
Catechol	2×10^{-4} *M*	63	62
Chlorogenic Acid	$1{\cdot}1\times10^{-4}$ *M*	100	—
Phloroglucinol	2×10^{-4} *M*	76	77
Hydroquinone	2×10^{-4} *M*	65	61
Dopa	1×10^{-4} *M*	—	63
Caffeic acid	$1{\cdot}1\times10^{-4}$ *M*	—	90
Catechol + Hydroquinone each	2×10^{-4} *M*	49	—
Tyrosine	$1{\cdot}1\times10^{-4}$ *M*	100	—
Resorcinol	2×10^{-4} *M*	100	—
2,4-dihydroxy benzoic acid	$1{\cdot}3\times10^{-4}$ *M*	100	—

It will be seen from this Table that a number of compounds are capable of producing an apparent loss of DPN. This appears to be due to a quenching effect in the case of catechol and phloroglucinol and hydroquinone. Whether quenching occurs or not is clearly related to the structure of the phenolic compound tested. It is particularly worth nothing that even compounds which fluoresce themselves such as DOPA and caffeic acid can cause an apparent reduction of the fluorescence of DPN in strong alkali. The fluorescence is clearly not additive.

It must be concluded that the method of LOWRY *et al.*[1] cannot be applied to any tissue where such phenolic compounds are likely to occur, especially since the amounts of and ratios between various phenols in plant tissues are liable to change markedly during development of the plant.

I would like to thank the Superintendent of the Low Temperature Research Station for providing the facilities for carrying out this work.

A. M. MAYER

Low Temperature Research Station, Cambridge, England, Dezember 8, 1958.*

Zusammenfassung

Die fluorometrische Bestimmung von Nukleotiden in Pflanzenextrakten wird durch phenolische Stoffe gestört. Nukleotide können mit dieser Methode nicht routinemässig bestimmt werden.

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[1] O. H. LOWRY, N. R. ROBERTS, and J. I. KAPPHANN, J. biol. Chem. *224*, 1047 (1957).

PRO EXPERIMENTIS

Vergleichende röntgenhistoradiographische und interferenzmikroskopische Trockengewichtsbestimmungen[1]

Von ENGSTRÖM und LINDSTRÖM[2] wurde die Röntgenhistoradiographie mit weichen Röntgenstrahlen zur Trokkengewichtsbestimmung an kleinsten biologischen Objekten eingeführt. Sie stellt neben der Interferenzmikroskopie eine wichtige unabhängige Methode für diese Zwecke dar. DAVIES *et al.*[3] untersuchten erstmals vergleichend mit beiden Verfahren verschiedene Objekte und fanden eine gute Übereinstimmung der Messergebnisse.

Wir benutzten in unseren röntgenhistoradiographischen Untersuchungen das Gerät von Philips (CMR 5). Wegen des kleinen ausgeleuchteten Feldes können hier Objekt und Referenzsystem kaum gleichzeitig photographiert werden. Wir mussten deshalb eine Methode entwickeln, die es gestattet, Trockengewichtsbestimmungen ohne Referenzsystem durchzuführen. Dazu wurde die Zeit-Schwärzungskurve für den verwendeten Film (linear bis $D = 0{,}7$ für Kodak Maximum Resolution Plates bei Entwicklung mit Gevaert G 209 A, 6 min, 18°C) bei konstanter Spannung festgelegt und mit einem Referenzsystem ein Trockengewichtsäquivalent bestimmt. (Für Einzelheiten der Methodik siehe MÜLLER *et al.*[4], im Druck.)

Die Objekte (Erythrozyten von *Rana temporaria*, Bullenspermien, Bullenthymuslymphozyten, Plattenepithelien der menschlichen Mundschleimhaut) wurden auf Prä-

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft.

[2] A. ENGSTRÖM und B. LINDSTRÖM, Nature *163*, 563 (1949); Biochim. biophys. Acta *4*, 351 (1950).

[3] H. G. DAVIES, A. ENGSTRÖM und B. LINDSTRÖM, Nature *172*, 1041 (1953).

[4] D. MÜLLER, W. SANDRITTER und G. SCHWAIGER, Histochemie (im Druck).

Vergleichende röntgenhistoradiographische interferenzmikroskopische Trockengewichtsbestimmungen

Zellart	Röntgenhistoradiographische Messungen		Interferenzmikroskopische Messungen	
	Zellzahl	Trockengewicht in 10^{-12} g	Zellzahl	Trockengewicht in 10^{-12} g
Bullenspermien	16	7,87 ± 0,46	162	8,94 ± 1,17 [5]
Bullenthymuslymphozyten	26	15,05 ± 0,53	96	14,40 ± 1,58 [5]
Froscherythrozyten				
Kern	49	24,00 ± 0,69	26	21,55 ± 0,84 [5]
Kern	18	21,29 ± 0,78	6	20,00 ± 0,72 [5]
Plasma	29	89,24 ± 5,40	–	– –
Plattenepithel der Mundschleimhaut				
Kern	5	77,80 ± 4,62	8	58,00 [8]
Kern	22	70,36 ± 4,26	38	55,50 ± 2,6 [6]
Plasma	27	1068,00 ± 84,00	8	2272,00 ± – [8]
Plasma	—	– –	37	1941,20 ± 61,5 [6]

[5] W. SANDRITTER, D. MÜLLER und H. G. SCHIEMER, Anat. Anz., 1959 (im Druck).

[6] W. SANDRITTER, H. G. SCHIEMER, W. ALT, D. MÜLLER und E. BEHROUZI, Frankf. Z. Path. *69*, 167 (1958).

parateträgern, die mit Mylarfolien bespannt waren, ausgestrichen. In allen Fällen, ausser den Mundschleimhautepithelien, kamen die gleichen Objekte mit beiden Methoden zur Untersuchung. Bei einer Spannung von 1,52 kV, 1,5 mA Röhrenstrom und 30 min Belichtungszeit wurden mit dem Historadiographen CMR 5 auf Kodak Max. Res. Plates Röntgen-Kontaktaufnahmen angefertigt und 6 min in G 209 A Gevaert bei 18°C entwickelt. Die Auswertung der Kontaktaufnahmen erfolgte mit einem Zytophotometer (SANDRITTER *et al.*[7]) mit einer Messblende von 5 μ^2. Die interferenzmikroskopischen Untersuchungen wurden mit der Analysatormethode durchgeführt (Bakersches Interferenzmikroskop, vgl. SCHIEMER *et al.*[8]).

Das Ergebnis der röntgenhistoradiographisch und interferenzmikroskopisch gewonnenen Trockengewichte zeigt die Tabelle. Die Messwerte zeigen eine gute Übereinstimmung. Die Abweichungen sind durch den systematischen Fehler beider Methoden bedingt. Diese Ergebnisse scheinen uns deshalb bemerkenswert, weil nicht nur die Genauigkeit der interferenzmikroskopischen und röntgenhistoradiographischen Technik aufgezeigt, sondern damit auch die von uns eingeführte Methodik der röntgenhistoradiographischen Trockengewichtsbestimmungen ohne routinemässige Verwendung eines Referenzsystems gesichert wird. So ist es erstmals möglich geworden mit dem einfachen Gerät CMR 5 quantitative Röntgenhistoradiographie bei geringem Arbeitsaufwand zu betreiben.

W. SANDRITTER und D. MÜLLER

Senckenbergisches Pathologisches Institut der Universität Frankfurt a. M., 10. November 1958.

Summary

Quantitative roentgen-histo-radiographic dry-weight determinations were made without a reference system. The results were compared to readings which had been obtained by the interference microscope. The readings from both methods correspond. The results show the range of error for both methods. It is remarkable that roentgen-histo-radiographic dry-weight determinations were made without using a reference system, once the dry-weight equivalent had been established.

[7] W. SANDRITTER, W. MONDORF, H. G. SCHIEMER und D. MÜLLER, Mikroskopie (im Druck).

[8] H. G. SCHIEMER, W. ALT und W. SANDRITTER, Acta Histochem. *4*, 325 (1957).

PRO EXPERIMENTIS

A Method of Reducing the Fibrinolytic Activity of Endometrium Cells in Plasma Clot Tissue Culture

The difficulty of preventing growing cells from digesting plasma coagel has been a problem since the earliest days of tissue culture. Repeated efforts have been made to overcome this obstacle (LOSEE and EBELING[1], CARREL[2]). More recently, the trypsin inhibitor, isolated by KUNITZ[3] from soya bean extract, has been used; but it was found to have a growth retarding effect and to prolong the coagulation time of the plasma (FISCHER[4]).

Certain cells, especially those of the uro-genital system, contain a large quantity of fibrinolysins; consequently the cells liquefy the plasma clot so much that there has been great difficulty in growing this kind of cell in tissue culture, even for short-term studies. In studying the endocrine reactions of rat endometrium *in vitro*, the present author met with this problem of preventing fibrinolysis. However, the addition of lysine-ethyl-ester (LEe) to the clot has made it possible to prevent its digestion by the endometrial cells. Experiments carried out by TROLL, SHERRY, and WACHSMAN[5] have shown that esters of lysine and arginine are specific substrates for plasmin, the proteolytic enzyme of mammalian plasma. Aware of this fact, the present author used the LEe in the plasma clot of her experiments to prevent the fibrinolytic action of the epithelial cells of rat endometrium.

Method. Small slices of uterus from albino rats, spayed 4–6 weeks before the experiments, were explanted in Carrel flasks (D 5) in a solid phase containing 0·5 ml of cockerel plasma and 0·5 ml of 5% chicken embryonic extract in Tyrode's solution. In some cultures LEe was added to the extract, producing a final concentration in the clot of 1·25–12·5 mg/ml. This solution, however, was found to be acid (10 mg/ml of LEe in Tyrode has a pH value of 6·4). Therefore, as a control, a Tyrode solution

[1] J. R. LOSEE and A. H. EBELING, J. exp. Med. *19*, 593 (1914).

[2] A. CARREL, J. exp. Med. *38*, 407 (1923).

[3] M. KUNITZ, Science *101*, 668 (1945).

[4] A. FISCHER, Science *109*, 611 (1949).

[5] W. TROLL, S. SHERRY, and J. WACHSMAN, J. biol. Chem. *208*, 85 (1954).