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THE DETERMINATION BY DIFFERENTIAL EXTRACTION OF THE DISTRIBUTION OF P^{32} LABEL IN SECTIONS AND SMEARS

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The incorporation and distribution of P^{32} label may be followed up by several biochemical methods [1, 2, 3]. The common principle in these methods is the determination of the specific activity of fractions obtained by differential extraction of homogenized tissues and cells. They do not allow, however, a histochemical or autoradiographic analysis of the material. Our recent researches into cellular metabolism have prompted the combination of the methods for determination of the P^{32} label with histochemical staining methods. The idea was to apply one of the biochemical methods in slides. On the basis of our trials the following procedure may be recommended.

Fresh frozen sections or isolated cells (protozoa, blood cells, spermatozoa, etc.) labelled with P^{32} are transferred to slides. The slides must be small so as to enable the radioactivity (expressed as count/minute) to be measured by an ordinary GM tube and scaler. The activity of the air-dried slides is measured and taken as the initial count. The P^{32} labelled compounds are then extracted by a modified Schmidt-Thannhauser procedure.

1. Fixation and removal of the acid soluble compounds (TCA-P) by cold (4°) 10% trichloroacetic acid (TCA) for 1 minute; thorough rinsing in tap water or 1% Na_2CO_3.

2. Extraction of the P-containing lipids (lipid-P) in several steps:
   a) treatment with 95% ethanol for 2×5 minutes,
   b) treatment with boiling ether-ethanol (1 : 1) for 5 minutes,
   c) rinsing in ether,
   d) treatment with boiling methanol-chloroform for 30 minutes.

Then either

3a. Extraction of the ribonucleic acid (RNA-P) by digestion with ribonuclease (RNase) at 37° for 18 hours. 0.5 mg/ml crystalline enzyme dissolved in distilled water;
   α) measurement of ultraviolet absorption of that solution before and after incubation and determination of its activity following incubation;

b. extraction of the deoxyribonucleic acid (DNA-P) by hot (90°) 5% perchloric acid (PCA) for 20 minutes;
   β) determination of its ultraviolet absorption and activity after treatment;

or

4. extraction of total nucleic acid (NA-P) by hot (90°) 10% TCA for 20 minutes.

The slides are dried after each step and their activity is measured. The data thus obtained correspond to the amount of the label retained in the slides. The amount of the label in individual fractions is expressed by the difference of the counts before and after extraction. All activities are given obtained in percentage of the initial activity. $3/\alpha$ and $3/\beta$ steps allow computation of the specific activity (expressed as Imp/min/1 γ NA) of P in both nucleic acids extracted.

Our method enables direct determination of the label in the following fractions: acid-soluble compounds, lipids, RNA, DNA; and determination of the specific activity of the phosphorus in the nucleic acids. The amount of the label in DNA is either determined by PCA treatment after RNase digestion (steps 3/a and 3/b) or computed as the difference of the means of NA-P and RNA-P (steps 3/a and 4). Most of the label retained (R—P) after the removal of total nucleic acid is probably bound to protein.

The slides may be treated by cytochemical methods or covered with autoradiographic emulsions so that the changes occurring during extraction may be followed up.

Tables I—IV give some examples of our results.

The method presents a picture of the distribution of the P^{32} label incorporated in cells or tissues (Tables I—III). Furthermore it facilitates comparison of the nucleic metabolism of the cells and tissues through the determination of the specific activities in the nucleic acids (see Table IV). In work performed on isolated cells, counting of the cells may lead to more conclusive results.

Table I

Distribution of P^{32} label in spermatozoa from bull epididymis incubated for 2.5 hours in Tyrode solution containing P^{32}

	No. of slide	TCA-P	Lipid-P	RNA-P	DNA-P	NA-P	DNA-P+R-P	R-P
			in per cent of initial count					
I RNase digestion	1	96.4	—	2.72	—	—	0.80	0.80
	2	94.5	—	4.58	—	—	0.83	0.83
	3	95.75	—	3.46	—	—	0.79	0.79
	4	94.8	—	4.42	—	—	0.78	0.78
	5	95.9	—	3.05	—	—	0.91	0.91
II TCA extraction of total NA	6	96.3	—	—	—	3.00	0.62	0.62
	7	96.5	—	—	—	2.75	0.73	0.73
	8	95.0	—	—	—	3.70	0.98	0.98
	9	95.4	—	—	—	4.46	0.54	0.54
	10	95.2	—	—	—	4.24	0.58	0.58
Mean		95.6	—	3.64	—	3.63	I 0.82 II 0.63	I 0.82 II 0.63
S. D.		± 0.708	—	± 0.824	—	± 0.755	± 0.029 ± 0.189	± 0.0529 ± 0.189

Table II

Distribution of P^{32} label in Tetrahymena pyriformis, *strain Gl cultivated for 48 hours in 1% peptone solution containing P^{32}*

No. of slide	TCA-P	Lipid-P	RNA-P	DNA-P	NA-P	DNA-P+R-P	R-P
	in per cent of initial count						
1	24.5	32.1	31.7	—	—	11.2	—
2	23.5	36.1	32.6	—	—	11.0	—
3	20.0	36.5	33.3	—	—	10.2	—
4	21.0	36.4	33.8	—	—	10.8	—
5	27.5	29.7	32.3	—	—	10.7	—
6	24.2	31.4	—	—	41.0	—	3.5
7	27.1	29.4	—	—	40.3	—	3.2
8	28.3	29.2	—	—	39.4	—	3.1
9	22.1	33.8	—	—	40.4	—	3.7
10	25.6	28.3	—	—	42.7	—	3.4
Mean	24.38	32.29	32.3	7.4	40.7	10.8	3.3
S. D.	± 2.77	± 3.20	± 0.96	—	± 1.022	± 0.37	± 0.25

Note: Mean of DNA-P computed as the difference of the means of NA-P and RNA-P. The difference of the means of DNA-P + R-P and R-P gives the same figure (7.5) for the DNA-P, which points to the correctness of the method.

Table III

Distribution of P^{32} label in isolated rat thymocytes 48 hours after intraperitoneal administration of labelled inorganic phosphate

No. of slide	TCA-P	Lipid-P	RNA-P	DNA-P	NA-P	R
	in per cent of initial count					
1	51.7	20.6	17.8	6.0	23.8	3.44
2	53.5	20.0	18.0	5.6	23.6	3.52
3	54.9	19.0	18.3	5.7	24.0	3.48
4	52.5	21.2	17.3	5.9	23.2	3.50
Mean	52.5	20.2	17.8	5.8	23.6	3.48

Note: The amount of DNA-P has been determined by its removal from all slides after the digestion with RNase (step 3b).

While the usual methods for the differential extraction of slides labelled with P^{32} aim at the removal of only one compound [4], our procedure permits the study of several fractions. The method may facilitate the use of P^{32} in autoradiography because slides containing the label in one certain compound may be processed.

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Table IV

Distribution of P^{32} label in fresh frozen sections of rat thymus and liver removed 28 hours after intravenous administration of labelled inorganic phosphate

	Thymus						Liver				
No. of slides	TCA-P	Lipid-P	RNA-P	DNA-P	R-P	No. of slide	TCA-P	Lipid-P	RNA-P	DNA-P	R-P
	in per cent of initial count						in per cent of initial count				
1	34.6	20.2	14.0	25.3	5.6	1	24.1	52.5	13.1	5.2	5.1
2	38.6	19.2	13.1	23.9	5.2	2	23.9	52.1	13.0	6.5	5.0
3	35.7	21.7	8.5	24.8	5.3	3	25.1	51.9	12.2	6.5	4.3
4	39.2	19.2	13.0	25.2	5.8	4	23.6	53.2	13.0	6.9	4.3
5	35.2	23.0	10.5	25.7	5.6	5	24.6	52.3	12.7	5.6	4.8
6	33.8	24.2	12.3	24.6	5.1	6	25.2	50.6	12.9	6.4	4.9
7	36.3	21.5	10.1	26.2	5.9	7	23.0	52.9	13.1	5.6	5.4
8	35.7	21.2	12.7	25.7	5.7	8	24.3	51.9	12.9	5.9	5.0
9	38.0	21.1	10.0	24.6	5.3	9	23.5	52.1	12.9	5.9	4.6
10	39.0	19.7	9.6	25.9	5.8	10	23.7	53.5	12.8	5.8	4.2
Mean	36.2	21.1	11.4	25.2	5.5		24.1	52.3	12.9	6.0	4.8
S. D.	± 1.99	± 1.62	± 1.85	± 0.71	± 0.28		± 0.7	± 0.81	± 0.27	± 0.52	± 0.4
Spec. act. (imp/min./1 γ NA)			15.6	9.9					24.6	1.2	

Note: Specific activities determined according to $3|\alpha$ and $3|\beta$.

Summary

A method has been outlined for determination of incorporation and distribution of P^{32} label in isolated cells or sections. Fresh frozen sections of tissues or smears of cells containing P^{32} are extracted according to a modified biochemical (Schmidt—Thannhauser) procedure. The amount of the label in individual fractions is computed from the counts obtained before and after the extractions. The analysis of the incubation solutions allows the determination of the specific activity of nucleic acid (RNA and DNA) phosphorus. The processed slides may be subjected to histochemical or autoradiographic procedures so that combined biochemical and morphological results are obtained.

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ОПРЕДЕЛЕНИЕ РАСПРЕДЕЛЕНИЯ P^{32} В СРЕЗАХ И МАЗКАХ ПУТЕМ ДИФФЕРЕНЦИАЛЬНОЙ ЭКСТРАКЦИИ

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Описывается метод для исследования включения и распределения P^{32} в изолированных клетках и в тканях. Свежие срезы приготовили из тканей назамораживающем микротоме. Из срезов или мазков клеток, содержащих P^{32} экстрагируются содержащие фосфор вещества по видоизмененному биохимическому методу Шмидта и Таннгаузера. Количество P^{32} в отдельных фракциях вычисляется из активности, установленной перед и после экстракции. Путем анализа экстрагирующих растворов определяется удельная активность фосфора нуклеиновых кислот (РНК—ДНК). Обработанные срезы и мазки разрешают гистологический или авторадиографический анализ для сравнения биохимических данных с морфологическими.

BESTIMMUNG DER VERTEILUNG VON P^{32} IN GEWEBESCHNITTEN UND AUSSTRICHEN DURCH DIFFERENTIALEXTRAKTION

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Es wird eine Methode zur Untersuchung der Inkorporation und Verteilung von P^{32} in isolierten Zellen oder Schnitten beschrieben. Frische Gefrierschnitte von Geweben oder Ausstriche von Zellen, die P^{32} enthalten, werden nach der modifizierten biochemischen Methode von Schmidt—Thannhauser extrahiert. Die Menge des P^{32} in den einzelnen Fraktionen wird aus der vor und nach den einzelnen Extraktionschritten gemessenen Aktivität berechnet. Eine Analyse der Inkubationsflüssigkeiten ermöglicht die Bestimmung der spezifischen Aktivität des Phosphors in den Nukleinsäuren (RNS und DNS). Zum Vergleich der biochemischen und morphologischen Ergebnisse können die so behandelten Schnitte oder Ausstriche auch histochemisch oder autoradiographisch untersucht werden.

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