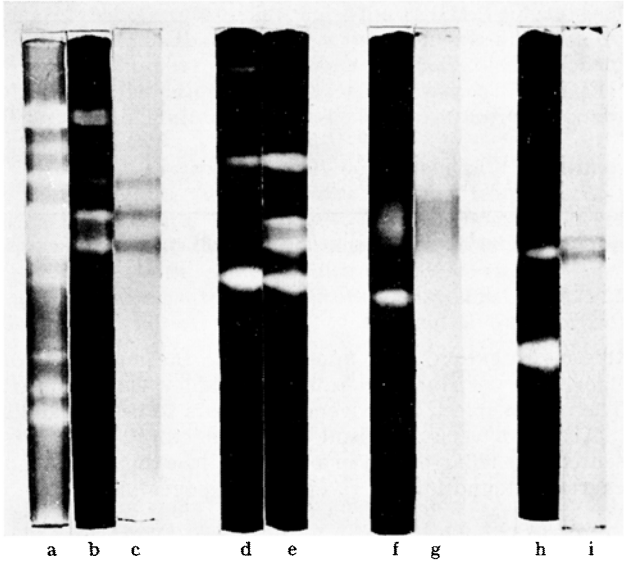
Für eine ausreichende Anfärbung waren mindestens 2 h erforderlich. Gewöhnlich aber wurden die Gele für ca. 12 h in der Farblösung belassen. Der nicht adsorbierte Farbstoff liess sich durch Waschen in 0,1prozentiger Essigsäure entfernen. Sobald die als helle Zonen erscheinenden RNase-Banden zu erkennen waren (nach ca. 12 h), wurde der Waschprozess abgebrochen. Durch längeres Auswaschen konnten in begrenztem Masse auch schwache Zonen noch sichtbar gemacht werden (Figur a).

Der Phosphodiesterase-Nachweis erfolgte nach LERCH[9].

Durch Zusammenpressen der Gele zwischen zwei Plexiglasplatten wurde die photographische Wiedergabe der Zonen verbessert.



a b c d e f g h i

Ribonukleasen und Phosphodiesterasen verschiedener Pflanzen nach Disk-Elektrophorese in Polyacrylamid-Gelen. (a) RNasen aus Weizenblättern. Nach dem Anfärben wurden die Gele ca. 24 h gewaschen. (b) RNasen aus Weizen. Die gleiche Trennung wie (a), jedoch wurden die Gele in diesem Fall nur ca. 12 h gewaschen. (c) Phosphodiesterasen aus Weizenblättern. (d) RNasen aus Kallus-Kulturen von Bohnen der Sorte Favorit. (e) RNasen aus Bohnenwurzeln der Sorte Favorit. (f) RNasen aus Bohnenwurzeln der Sorte Red Kidney. (g) Phosphodiesterase aus Bohnenwurzeln der Sorte Red Kidney. (h) RNasen aus Zuckerrübenblättern. (i) Phosphodiesterasen aus Zuckerrübenblättern.

Ergebnisse und Diskussion. Ähnlich wie für andere Enzyme (vgl. MAURER[10]) konnten auch für RNasen Unterschiede in Zahl und Position der Zonen zwischen verschiedenen Pflanzengattungen, -sorten und auch -geweben gleichen genetischen Ursprungs festgestellt werden.

So wurden nach Inkubation bei pH 5 für Weizenblätter 11, für Bohnenwurzeln der Sorte Favorit 4, für Kallusgewebe der gleichen Sorte 3, für Bohnenwurzeln der Sorte Red Kidney 2 und für Zuckerrübenblätter 3 Zonen nachgewiesen (Figur a, b, d, e, f, h). Grundsätzlich das gleiche Bild ergab sich, wenn die Gele bei pH 7,5 inkubiert wurden, jedoch deutet die unterschiedliche Stärke der Banden auf verschiedene pH-Optima der einzelnen Enzyme hin.

Von den RNS-spaltenden Enzymen haben beim Weizen 3, bei Bohnen der Sorte Red Kidney 1 und bei Zuckerrüben 2 Phosphodiesterase-Eigenschaften (Figur c, g, i).

Im Vergleich mit dem bisher am häufigsten verwendeten, indirekten Nachweis von Nukleasen führt die hier beschriebene Methode zur Ausbildung schärfer abgegrenzter Zonen; gegenüber dem von BOYD und MITCHELL beschriebenen Verfahren hat sie den Vorteil, einfacher und weniger kostspielig zu sein. Durch Kombination mit dem Phosphodiesterase-Nachweis nach LERCH[9] bietet sie zudem die Möglichkeit, Nukleasen nach ihrem Spaltungsmechanismus zu differenzieren[11,12].

Summary. Detection of ribonucleases in polyacrylamide gels after disc-electrophoresis is possible by incubation of the gels in a solution of low molecular RNA followed by staining with methylene blue. Application of this method to protein extracts of wheat leaves, sugar beet leaves and roots of different bean varieties as well as callus cultures and roots of the same bean variety shows differences in number and position of RNase zones.

G. WOLF

Institut für Pflanzenpathologie und Pflanzenschutz der Universität, 34 Göttingen (Deutschland), 13. Mai 1968.

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Effect of β-Aminoethylisothiuronium-bromide-hydrobromide on the Acetate-1-^{14}C Incorporation into Tissue Lipids of Irradiated Rats

X-irradiation in rats is known to lead to increased synthesis of fatty acids from acetate in liver[1-6] and kidney[7]. Disturbances in the lipid levels of tissue have been reported[8] as a result of whole-body γ-irradiation and also after β-Aminoethylisothiuronium-bromide-hydrobromide (AET) administration. Disappearance of epidymal fat pads was noted 48 h after 2400 R irradiation in early stages of this study. The adipose tissue is an important site for synthesis of triglycerides from carbohydrates and other 2-carbon precursors[9,11]. In irradiated rats the disappearance of this important site of lipogenesis would result in an increased out-put of lipids by tissues less important for lipogenesis, in the presence of adipose tissue. Liver and kidney synthesize lipids to a smaller extent in normal rats. With a view to evaluate relative contribution of different tissues, in irradiated and AET treated rats, in vivo acetate-1-^{14}C incorporation into lipids was studied.

Methods and materials. Young male albino rats weighing 100–110 g were divided into 4 groups with 4 animals in each group. Rats of group A were sham-irradiated and served as controls. Animals of group B were subjected to 2400 R whole body γ-irradiation in a γ-cell from all the sides. Rats of group C and D were injected with a neutral

Effect of irradiation and AET-administration on tissue weight, total lipids acetate-1-^{14}C incorporation into lipids of rat tissues

Groups	Tissue weight	Liver			Kidney			Spleen	
	g/100 g body weight	Total lipids mg/g tissue	Radio-activity cpm/mg lipid	Tissue weight g/100 g body weight	Total lipids mg/g tissue	Radio-activity cpm/mg lipid	Tissue weight g/100 g body weight	Total lipids mg/g tissue	Radio-activity cpm/mg lipid
Control (A)	2.82 ± 0.08	62.41 ± 3.09	241 ± 19 (100)	0.882 ± 0.024	64.73 ± 4.47	122 ± 14 (100)	0.301 ± 0.031	35.42	311 (100)
Irradiated (B)	4.08 ± 0.33	48.23 ± 1.99	1089 ± 94 (452)	0.882 ± 0.012	39.97 ± 2.32	266 ± 15 (218)	0.093 ± 0.004	82.66	493 (158)
Irradiated + AET (C)	3.12 ± 0.17	60.58 ± 5.66	695 ± 61 (288)	0.868 ± 0.056	61.96 ± 1.80	124 ± 11 (102)	0.096 ± 0.005	70.55	243 (78)
Control + AET (D)	3.04 ± 0.13	76.28 ± 11.01	199 ± 33 (83)	0.682 ± 0.021	61.36 ± 2.43	70 ± 14 (57)	0.246 ± 0.010	33.34	245 (78)
Between groups	Significance								
A and B	< 0.01	< 0.05	< 0.01	-	< 0.01	< 0.01	< 0.01		
A and C	< 0.01	> 0.80	< 0.01	> 0.9	> 0.50	> 0.90	< 0.01		
A and D	> 0.5	> 0.30	> 0.30	< 0.01	> 0.50	> 0.05	> 0.2		
B and C	< 0.05	> 0.05	< 0.05	> 0.9	< 0.01	< 0.01	> 0.9		

Each value for liver and kidney is the mean ± S.E. of the result of 4 separate experiments and for spleen is the result of 4 pooled samples. $p \leqq 0.05$ has been considered significant.

solution of AET (0.2 mg/100 g body weight i.p.). 10 min later the rats of group C were irradiated as in the case of the rats of group B.

All the rats were fed ad libitum until about 1 h before irradiation. No food was allowed from the time of irradiation to the time of sacrifice. Animals had free access to water throughout the experiments. The animals were injected with 10 μc sodium acetate-1-^{14}C/100 g body weight i.p. 46 h after irradiation. The rats were sacrificed 48 h after irradiation by decapitation. The tissue lipid extract was prepared as described earlier[10]. Total lipids were determined gravimetrically. Samples of total lipids were pipetted in glass vials and dried at 80 °C. The residue was dissolved in 10 ml scintillation fluid (4 g 1,4-*bis*-2-(5-phenylorazolyl-benzene) and 10 mg 2,5-diphenylorazole dissolved in 1 l of extrapure toluene). The samples were counted in a Packard Tricarb liquid scintillation counter fitted with an automatic recorder. The recounts were corrected for background counts as well as for quenching. The counting efficiency of the system was 70%.

Results and discussion. Acetate-1-^{14}C incorporation into lipids is increased in liver, kidney and spleen (Table), 48 h after 2400 R whole body γ-irradiation. However, the increase varies from tissue to tissue. In liver, the increase in acetate incorporation as a result of irradiation is 452% of control. In AET-treated irradiated rats the increase is only 288% showing that AET affords some protection. In AET-treated controls the incorporation is decreased to 83%. In kidney, acetate incorporation is increased to 218% after irradiation and AET affords complete protection against this increase. AET-treatment of controls decrease acetate incorporation to 57%. Lipogenesis in spleen appears to be least affected and acetate incorporation is increased to 158% only. Both AET-treated irradiated and AET-treated control rats show a decreased incorporation to 78%. Tissue weight/100 g body weight of liver is increased significantly, that of kidney is unchanged and that of spleen reduced significantly (Table). In the light of the above observations, among the tissues studied in this investigation, liver appears to be the major site of lipogenesis in irradiated rats with kidney coming next. The role of spleen in lipogenesis appears to be negligible.

AET is a very promising radioprotector[12]. Data presented here indicate that in a very low dose this compound exerts profound inhibitory effect on lipogenesis.

Zusammenfassung. Einfluss von Bestrahlung auf die Lipidsynthese, wobei festgestellt wird, dass der Strahlungsschutzstoff AET die Lipidsynthese hemmt.

S. NAQVI, K. S. KUMAR and T. A. VENKITASUBRAMANIAN

Department of Biochemistry, V. Patel Chest Institute, University of Delhi, Delhi 7 (India), 22 March 1968.

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