row proteins in *Triturus* tissue during the cultivation from 1–3 h. The titer is seemingly affected by newt proteins in solution, which might explain the high titers observed in experimental series compared to the titers observed by adjusting the antiserum. It has been noted before that to obtain some mesodermal induction, a bone marrow implant must act on ectoderm for at least 3 h[8]. Thereafter the degree of induction shows only slight increase. Even if our results do not justify correlation of the movement of antigens to the primary induction phenomenon, they offer two important suggestions. First, it seems probable that the handling of inductor tissue with 70% alcohol, even when denaturing the greater part of soluble proteins, leaves certain antigenic material active and this may pass into the reaction material during the time primary induction has been assumed to occur. Secondly, the same kind of movement of antigens as observed by FLICKINGER[6] can be demonstrated when using 'chimeric explants' of fixed adult heterogenous tissues and newt totipotent ectoderm. The residual inductor material after removal of implant has been considered minimal[8]. This suggestion is supported by the fact that, in a number of slides stained with fluorescent antibodies, the fluorescence was observed to be localized to the ectodermal cells and not to extracellular material.

The results, which have been confirmed using fluorescent antibodies in preliminary experiments, open up possibilities for closer analysis of transferred material during primary induction; adult antigens provide a good tool for preparing efficient antibodies and it has been noted previously that the effect of mesodermal inductor might be inhibited by antibodies against inducing tissues[9].

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State Serum Institute, Helsinki, Department of Pathology, Section II and Zoological Laboratory, University of Helsinki (Finland), September 9, 1959.

Zusammenfassung

Der Übertritt von Antigenmaterial aus heterogenem Induktor in Ektoderm wurde mit der Ringpräzipitation untersucht. Nach Entfernung des Implantates wurde als Antigen ein Ektodermextrakt verwendet und als Induktor alkoholbehandeltes Knochenmark von Meerschweinchen. Die ins Ektoderm übergetretene Antigenmenge war bei 3 h alten Kulturen grösser als bei 1 h alten.

Addendum: Since this preliminary note was sent to the editor, a paper has appeared[10] showing the transfer of guinea pig antigenicity from the liver cells (treated with alcohol) to the totipotent ectoderm. The fluorescent antibody technique was used and the explants were prepared from *Triturus alpestris* ectoderm.

[8] S. TOIVONEN, J. Embryol. exp. Morph. *6*, 479 (1958).

[9] T. VAINIO, Exp. Cell Res. *15*, 184 (1958).

[10] R. M. CLAYTON and A. ROMANOVSKY, Exp. Cell. Res. *18*, 410 (1959).

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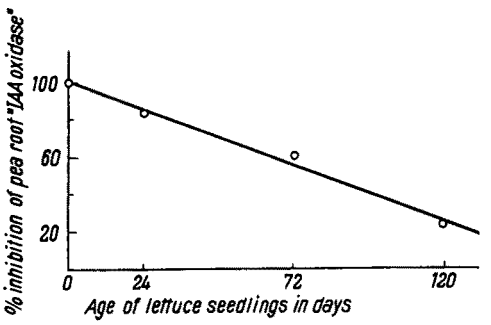
A Thermostable Substance from Lettuce Seeds and Seedlings Inhibiting β-Indolyl-acetic Acid Oxidation

During the course of an investigation into the growth substances present in lettuce seeds and seedlings (variety Grand Rapids), the IAA[1]-oxidizing activity of homogenates of these tissues was tested. The presence of 'IAA oxidase', i.e. the complex of enzymes responsible for the oxidation of the auxin[2], could not be demonstrated either in ungerminated seeds or in seedlings up to the age of 20 days. A thermostable inhibitor (or inhibitors) of IAA oxidation was, however, detected in homogenates of both



The relationship between the age of lettuce seedlings extracted and the degree of inhibition of 'IAA oxidase' produced by the extracts (Lettuce seedlings were germinated in complete darkness).

seeds and seedlings. This inhibitor was tested on an IAA-oxidizing system prepared from roots of 8-day old etiolated Alaska pea seedlings. 100 g (fresh weight) of root tissue was ground in 100 ml 0·15 *M* phosphate buffer, pH 6·0. Cell debris was removed by squeezing through cheese cloth and centrifuging at 700 × g for 5 min. The supernatant served as the source of the IAA oxidase. Lettuce homogenates were prepared in a similar way, by grinding 1 g of seeds or seedlings in 10 ml of phosphate buffer pH 6·0. Three ml of the IAA oxidase suspension were mixed with 1–3 ml of lettuce homogenate, 1 ml 10^{-4} *M* $MgCl_2$, and 0·5 ml 10^{-3} *M* IAA. The reaction mixture was then made up to 10 ml with phosphate buffer and allowed to incubate for 1 h at 26°C in the dark. Residual IAA was estimated by the method of TANG and BONNER[3] after the reaction mixture had been cleared with 30% trichloracetic acid (TCA). Preliminary experiments[4] showed that addition of TCA to the reaction mixture increased the intensity of the colour developed with the reagent. Calibration curves containing the appropriate amount of TCA were therefore prepared.

The effect of dialysis on the degree of inhibition produced by homogenates of lettuce on pea root 'IAA oxidase'

Age of seedlings in days	% inhibition of pea root 'IAA oxidase'		
	Before dialysis	After dialysis	
		24 h of dialysis	84 h of dialysis
0	100	60	—
24	84	52	50
35	68	46	—

It will be seen in the Figure that IAA oxidation was entirely prevented in the presence of 1 ml of a homogenate of ungerminated lettuce seeds. As germination

[1] IAA = β-Indolyl-acetic acid.

[2] W. D. BONNER, JR., Ann. Plant. Physiol. *8*, 427 (1957).

[3] J. W. TANG and J. BONNER, Arch. Biochem. *13*, 11 (1947).

[4] S. BLUMENTHAL-GOLDSCHMIDT, Ph. D. Thesis, Hebrew University, Jerusalem.

progressed, the inhibitory power of the homogenate declined (Figure). Heating the homogenate in a boiling water-bath for 10 min did not diminish its inhibitory activity. Dialysis through a cellophane membrane against 0·15 *M* phosphate buffer resulted in a partial removal of inhibitory activity (see Table). However, even prolonged dialysis (48 h) against constantly renewed buffer solution failed to remove the inhibitory material entirely, suggesting that more than one substance may be involved in this inhibition.

Even after prolonged dialysis, no IAA-oxidizing activity could be detected in the lettuce homogenates.

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Résumé

Des extraits de graines et de plantules de laitue (*Lactuca sativa*) var. Grand Rapids inhibent l'auxine-oxydase des homogénats de racine de pois Alaska. L'effet inhibiteur de ces extraits de laitue n'est pas détruit par la chaleur, et n'est que partiellement réduit par dialyse.

Inhibition of 5-Hydroxytryptamine Release from Blood Platelets by N²-Isopropyl Isonicotinic Acid Hydrazide

It has been shown that reserpine and 2-oxo-3-isobutyl-9, 10-dimethoxy-1, 2, 3, 4, 6, 7-hexahydro-benzo[a]quinolizine[1] cause a decrease of 5-hydroxytryptamine (5HT) in isolated blood platelets of rabbits[2]. Thereby the concentration of the free 5HT in the plasma increases if the animals have been pretreated with a monoamine oxidase (MAO) inhibitor [1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine[3]]. This probably shows that although MAO is inhibited, reserpine and the benzoquinolizine derivative are still capable to release 5HT from platelets. On the other hand there exists some evidence that in the intestine and the brain the MAO inhibitor N²-isopropyl isonicotinic acid hydrazide (iproniazid)[4]) inhibits the reserpine induced release of 5HT from tissue structures[5]. A definite proof for this hypothesis has, however, not been presented up to now.

The following work was undertaken to investigate whether iproniazid inhibits the reserpine induced 5HT release in isolated blood platelets.

Method. Using a procedure previously described[6] 5HT determinations in platelets and platelet poor plasma were carried out after incubation of platelet rich rabbit plasma with reserpine for 3 h. Part of the samples were incubated with iproniazid alone or with iproniazid and reserpine (reserpine added 15 min after iproniazid, final iproniazid

[1] Trade name Nitoman.

[2] A. CARLSSON, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. exp. Ther. *120*, 334 (1957). – G. P. QUINN, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. exp. Ther. *127*, 103 (1959). – M. K. PAASONEN and A. PLETSCHER, Exper. *15*, [MS no. 343, Dez.-Heft] (1959).

[3] Trade name Marplan.

[4] Trade name Marsilid.

[5] G. ZBINDEN, A. PLETSCHER, and A. STUDER, Klin. W'schr. *35*, 565 (1957). – N. J. GIARMAN and S. SCHANBERG, Biochem. Pharmacol. *1*, 301 (1959).

[6] M. K. PAASONEN and A. PLETSCHER, Exper. *15*, MS no. 343 Dez.-Heft (1959).

Incubation	Undiluted platelet suspension (platelet rich plasma)			Diluted platelet suspension			Significance *p*
	No Iproniazid (1)	With Iproniazid (2)	% Difference (3)	No Iproniazid (1)	With Iproniazid (2)	% Difference (3)	
None	12·7	11·8	− 7	13·4	11·8	−12	
	13·6	13·6	0	7·7	7·8	+ 1	
	11·1	10·8	− 3	12·3	12·6	+ 2	
	18·6	19·4	+ 4				
Mean	14·0 ± 1·7 VI	13·9	−1·5 ± 2·5 I	11·1	10·7	− 3 ± 4·5 II	
							III/I < 0·01
	6·3	7·4	+18				IV/I < 0·01
Reserpine	5·5	8·6	+57				V/I < 0·01
1 μg/cm³	1·5	2·4	+60				V/II < 0·01
	11·6	15·3	+32				V/IV > 0·05
	8·8	10·8	+23				I/II > 0·05
							VI/VII < 0·01
Mean	6·7 ± 1·6 VII	8·9	+38 ± 8·5 III				VI/VIII < 0·01
	11·0	12·0	+ 9	12·5	14·0	+12	
Reserpine	6·5	7·8	+20	3·6	4·9	+36	
0·3 μg/cm³	8·2	10·8	+32	7·6	8·7	+15	
				5·7	7·8	+37	
Mean	8·6 ± 1·3 VIII	10·2	+20 ± 6·5 IV	7·4	8·9	+25 ± 6·5 V	

5HT content of rabbit platelets with and without iproniazid preincubation *in vitro*. The figures in columns 1 and 2 indicate the 5HT in μg contained in platelets from 1 cm³ platelet rich plasma. Reserpine incubation for 3 h; addition of iproniazid 1/4 h before reserpine. Standard errors.