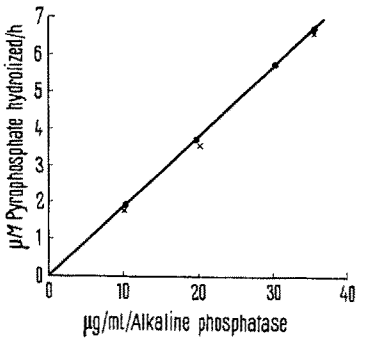
pyrophosphate, or condensed phosphates in general (the authors used Graham salt), 'prevent the initiation and/or growth of calcium phosphate crystals by acting directly at what would otherwise be a site of calcification'. In their experiments the possibility can be excluded that polyphosphates act by lowering the extracellular concentration of biologically active calcium by binding this mineral in a complex form. The presumptive local inhibitory action of pyrophosphate on calcium phosphate precipitation could be demonstrated in the third group of our experimental animals. It was demonstrated that the inhibitory action of pyrophosphate on calcium phosphate precipitation could be destroyed by alkaline phosphatase. This enzyme proved to be capable of hydrolyzing pyrophosphate to orthophosphate (the Figure shows this hydrolytic activity). 16 U of alkaline phosphatase (Böhringer, Mannheim[7]) proved to be capable of hydrolyzing 7.2 mg of pyrophosphate. In our experiments the local concentration of alkaline phosphatase was, therefore, high enough to hydrolyze all of the pyrophosphate available for the inhibition of calcification.



Hydrolytic activity of alkaline phosphatase on pyrophosphate. TRA-buffer 0.1 *M*, pH 7.0; pyrophosphate 6,6 μM, 37 °C.

Injection of pyrophosphate in an amount sufficient to prevent heterotopic skin calcification has no influence on the formation and growth of normal bone. Treatment of normal growing rats with pyrophosphate does not affect the final size and weight of these animals when compared with control rats receiving sham injections of 0.9% saline instead of pyrophosphate (author's unpublished experiments). We are convinced that in normal bone and epiphyseal plate there is enough alkaline phosphatase present to hydrolyze all pyrophosphate and, thereby, destroy this calcification inhibitor.

The most probable local action of pyrophosphate inhibition consists of an interference with the initial step of calcification – the binding of collagen and phosphate. It may be that a collagen-pyrophosphate binding occurs instead of the normal collagen-phosphate binding.

Zusammenfassung. Durch $KMnO_4$-Injektionen hervorgerufene lokale Hautverkalkung kann durch Pyrophosphatinjektionen verhindert werden. Durch alkalische Phosphatase wird die Pyrophosphatwirkung aufgehoben.

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I. Medizinische Klinik im Klinikum Mannheim der Universität Heidelberg and Abteilung für Medizinische Forschung der C. F. Böhringer, Mannheim (Germany), 28th October 1966.

[7] Alkaline phosphatase acetone dry powder from calf intestine, 8 U/mg, *p*-nitrophenylphosphate-substrate.

Effect of Irradiation on the Properties of Pollen in Austrian and Scotch Pines

It is known that several pine species belonging either to the same or different branches of the same subgenus cannot be mutually crossed. Such incompatibility exists, for instance, between the Austrian pine (*Pinus nigra* Aru.) and the red pine (*Pinus resinosa* Ait.) and between the Austrian pine and Scotch pine (*Pinus silvestris* L.). The problem of incompatibility between red pine and Austrian pine was studied by MCWILLIAM[1], who analysed sugars and amino acids in the ovules of these pines.

VIDAKOVIĆ[2], in a bid to overcome the incompatibility in the crossing of Scotch and Austrian pines, irradiated the pollens just prior to pollination, on the assumption that certain changes that were apt to occur in the irradiated pollens would help to circumvent the 'incompatibility barrier', resulting in the fecundation of the female flower of other species.

However, the effects of such a mutagen causing cytogenetic damage or modification are apt to be reflected also in the pollen biochemistry, i.e. sugars, amylase, amino acids, etc., present in mature pollens, are likely to undergo changes with irradiation. The present communication is concerned with the determination of sugars, amylase, and free and bound amino acids in irradiated as well as non-irradiated pollens of Austrian and Scotch pines with a view to demonstrating the differential effects of irradiation on the pollen of the 2 pine species.

Collection and irradiation of pollen. The male flower-branches were collected 1 day prior to pollen dusting. The pollen was collected after 24 h and dispersed pollen irradiated by means of a Co^{60} source of about 60 Curies. The irradiation doses were 200, 800, 1000 and 1200 r.

Total reducing sugar was determined by the SCHOORL-LUFF[3] method after the extraction of sugars from pollen with water.

Individual sugars. Qualitative determination of the individual sugars has been made by paper chromatography using the descending technique in the solvent system *n*-butanol-pyridine-benzene-water (5:3:1:5). The time of development of the chromatogram was 48 h. Detection was carried out by 2,3,5-triphenyl-tetrazolium chloride.

[1] J. R. MCWILLIAM, Am. J. Bot. *43*, 6, 425 (1959).
[2] M. VIDAKOVIĆ, World Consultation on Forest Genetics and Tree Improvement, Stockholm. F.A.O.For.Gen.-63, Vol. I, 2b/5:1–5 (1963).
[3] N. SCHOORL, Zeitschrift für Chemie *57*, 566 (1929).

The chromatogram showed higher % of fructose, comparatively lower % of glucose and maltose, and 5 unidentified oligosaccharides.

Amylolytic activity has been determined according to Wohlgemuth. Extraction of amylase from pollen was carried out by 0.2% NaCl solution. Values given in Wohlgemuth units stand for the number of ml of 1% soluble starch which is hydrolysed by 1 g of pollen to standard dextrin during 30 min at pH 7 and 38 °C. It is interesting that starch has not been detected in the pollens.

Amino acids. (a) Free amino acids: 1 g of pollen was continuously extracted with 50 ml of 70% ethanol during 24 h and the filtrate concentrated to 5 ml. (b) Bound amino acids: after the extraction of free amino acids, the residue was hydrolysed to liberate the bound amino acids. 0.5 g of dried residue of pollen was heated in ampoules with 6*N* HCl at 105 °C for 20 h. After the removal of the excess HCl acid by repeated evaporation on a water-bath, the liberated α-amino acid hydrochlorides were quantitatively dissolved in 10% iso-propanol.

For the qualitative separation of free and bound amino acids, the ascending chromatographic technique on Whatman paper No. 1 in the solvent system *n*-butanol-acetic acid-water (4:1:1) was applied.

Quantitative determination of amino acids was carried out by thin-layer chromatography[4,5] in several solvent systems on 2 plates simultaneously. 1 of the plates was prepared by applying a layer of silica gel mixed with phosphate buffer (pH 6.5). The separation of amino acids was performed in the system tertiary butanol-methyl-ethyl ketone-water (4:4:2). Then the dried plate was placed into the second system, butanol-acetic acid-water (4:1:1). The length of the displacement of the solvent front was 14 cm. The second plate was placed in the system chloroform-methanol-ammonia 25% (2:2:1).

Quantitative determination of amino acids was carried out in two ways: (a) by measuring the density of the spots on the plate directly, and (b) eluting the spots from the plate and reading the extinction in the spectrophotometer. A mixture of known amino acids was always used for reference on the same plate from which the corresponding amino acids from the sample (prepared from pollens) were eluted; the theoretical values for Rf were not used. The known standards showed simultaneously the position of the corresponding amino acids in the sample.

Results and discussion. Free and bound amino acids have been determined for irradiated as well as non-irradiated pollens of both the pine species. The results are shown in Tables I and II. It is apparent from Table I that the pollens of the 2 pine species do not differ remarkably, either in the amino acid composition or in the free amino acid content. But the differences in the bound amino acid content are more pronounced, though irregular. However, irradiation did not have any marked effect on the amino acid content of pollens of the 2 pine species within the radiation dosage applied, though an insignificant and irregular variation was noticed (Table II).

It is evident from the Figure that the concentration of sugar in the pollen of Scotch pine is more than double the concentration in the pollen of Austrian pine. It is also evident that irradiation has remarkably influenced the amount of amylase enzyme in the pollen, and it increases with increasing radiation showing a maxima at 1000 r; at the same radiation dosage the minima for the reducing sugars also occurs. It is significant that though the pollens

Table I. Free and bound amino acids in the pollen of Austrian and Scotch pines

Name of amino acid	Amino acids in the pollen of Austrian pine		Amino acids in the pollen of Scotch pine	
	mg/g of pollen			
	bound	free	bound	free
α-Alanine	11.10	0.70	5.20	0.45
Arginine	24.80	3.25	16.80	4.10
Asparagine	23.20	2.00	26.70	2.80
Cysteine	8.00	0.60	2.00	0.80
Phenyl alanine	4.50	–	4.00	–
Glutamic acid	26.50	2.10	16.80	1.30
Lysine	9.45	1.84	9.20	1.56
Leucine	9.70	–	4.61	0.70
Methionine and valine	6.70	0.40	4.01	0.60

Proline, tyrosine and tryptophan have also been found to be present in traces. The figures in the Table are the averages of 50 experiments performed during 5 years at the time of pollen dispersal.

[4] E. Mutschler and H. Rochelmeyer, Arch. Pharm., Berl. *492*, 449 (1959).

[5] A. R. Fahmy, A. Niederwiesser, G. Pataki and M. Brenner, Helv. chim. Acta *44* II, 2022 (1961).

Table II. Free and bound amino acids in the irradiated and non-irradiated pollen of Austrian and Scotch pines

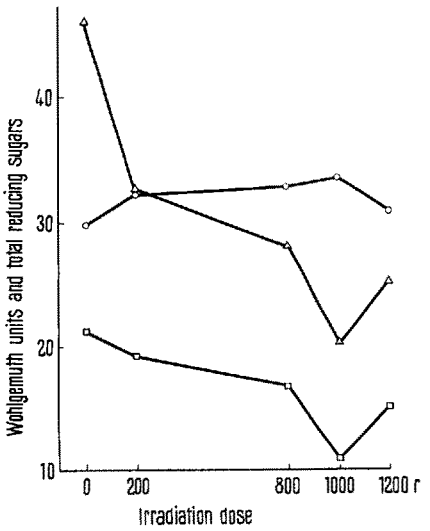
Name of amino acid	Amino acids in the pollen of Austrian pine								Amino acids in the pollen of Scotch pine							
	mg/g of pollen															
	unirradiated		200 r		800 r		1200 r		unirradiated		200 r		800 r		1200 r	
	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free	bound	free
α-Alanine	11.10	0.70	10.80	0.80	9.90	0.60	11.00	0.80	5.20	0.45	5.71	0.40	4.90	0.40	5.41	0.30
Glutamic acid	26.50	2.10	24.30	1.90	24.10	2.30	23.80	2.00	16.80	1.30	16.30	1.50	15.70	1.20	16.00	1.60
Lysine	9.45	1.84	9.35	1.80	8.91	1.95	8.20	1.72	9.20	1.56	9.20	1.62	8.40	1.50	9.60	1.56
Methionine and valine	6.70	0.40	7.40	0.40	6.40	0.40	6.90	0.50	4.01	0.60	4.40	0.50	3.91	0.60	3.74	0.60

The figures in the Table are the average of 50 experiments performed during 5 years at the time of pollen dispersal.

of Austrian pine contain 21.23 mg/g of reducing sugars while those of Scotch pine contain more than double that amount (46.05 mg/g), reducing sugars in the latter readily drop to 20.10 mg/g, almost equalling the amount of sugar present in the pollens of Austrian pine, when irradiated with 1000 r.

Obviously, so far as the chemical composition of the pollen is concerned, the Austrian and Scotch pines differ mainly in the amount of reducing sugar present, which is most likely to be a definite contributing factor towards the incompatibility of the 2 species; because VIDAKOVIĆ has been successful in bringing about radiation-induced hybridization of the aforesaid 2 pine species, irradiating the pollen of the Scotch pine between 800 and 1200 r prior to pollination.

However, it is too early to arrive at any definite conclusion as to the nature of these resulting hybrids. But a relationship between successful crossability and the amount of reducing sugar in the pollens could be discerned, and this finding might eventually prove significant in overcoming interspecific barriers[6].



o Wohlgemuth units in 1 g of pollen in Austrian pine. Total reducing sugars in mg/g of pollen: □ Austrian pine, △ Scotch pine.

Zusammenfassung. Kohlenhydrate, amylolytische Aktivität und Aminosäuren wurden in nicht bestrahlten und bestrahlten Pollen der gemeinen Kiefer und Schwarzkiefer bestimmt. Die Bestrahlung bis zu 1200 r hatte keinen signifikanten Einfluss auf den Gehalt an Aminosäuren, doch kann die Bestrahlung die Menge der reduzierenden Zucker bedeutend verändern. So kam es zur Ausgleichung des Zuckergehalts des unbestrahlten gemeinen Kieferpollens mit dem Zuckergehalt der bestrahlten Schwarzkiefer bei 1000 r. Unter diesen ähnlichen Bedingungen gelang die Befruchtung der genannten Kiefern.

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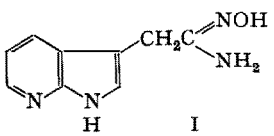
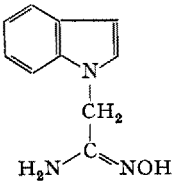
[6] This investigation was supported partly by grants from the Yugoslav Federal Nuclear Energy Commission and the Federal Research Work Fund.

Antihypertensive Activity of 7-Azaindole-3-Acetamidoxime and Indole-1-Acetamidoxime

The antihypertensive properties of 7-azaindole-3-acetamidoxime (I) and indole-1-acetamidoxime (II)[1] were studied in the renal hypertensive rat and dog and the normotensive rat, cat and dog. Dosages found to reduce blood pressure to normotensive levels in 50% of the hypertensive rats ($ED_{50} \pm$ S.E.) following oral administration were: (I) 14.2 ± 3.1 mg/kg, (II) 18.2 ± 4.5 mg/kg; and following s.c. injections were: (I) 15.2 ± 3.3 mg/kg, (II) 13.8 ± 1.8 mg/kg indicating effective oral absorption with both compounds. Oral administration, once daily, in the hypertensive dog produced a gradual, sustained lowering of blood pressure to normotensive levels without side effects at 2–4 mg/kg with either I or II. Maximum lowering of blood pressure was observed at 3 days and return to hypertensive levels after discontinuing medication occurred in 3–4 days. Bradycardia was observed during the antihypertensive response in the dog with I but no change in heart rate was observed with II. Neither compound lowered the blood pressure of the normotensive rat or dog.



I



II

In the pentobarbitalized normotensive cat and dog, both I and II at doses of 2.5–10 mg/kg produced a marked vasopressor response beginning in 2–3 min and lasting 90–180 min with a secondary fall in blood pressure after about 300 min with I but not with II. The vasopressor response with both compounds was abolished by pretreatment with phentolamine. Both I and II inhibited the vasopressor response to bilateral carotid occlusion and injected epinephrine and norepinephrine. Reversal of the vasopressor response to injected epinephrine was observed

[1] The subject compounds and others related are described in Sterling Drug Inc., Dutch Patent 6,501,742 (1965).