Chromatographie unter Verwendung von Vergleichssubstanzen identifiziert werden[7].

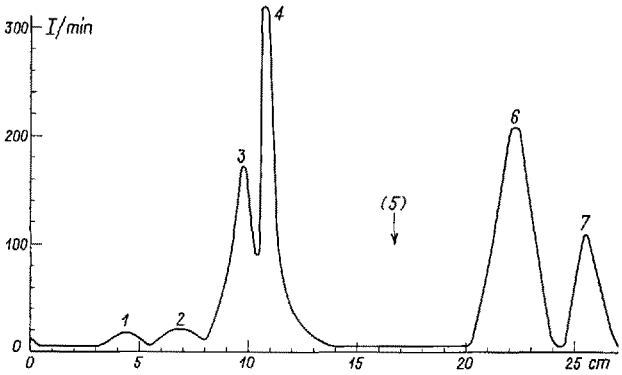

Abb. 2. Radiochromatogramm des Proteinhydrolysats von *Fusarium lycopersici* (Phenol-Wasser-Ammoniak). Abszisse: Entfernung vom Startpunkt. Ordinate: Impulse pro Minute. *1* Asparaginsäure; *2* Glutaminsäure; *3* Serin; *4* Glykokoll; *6* Leucin; *7* Valin. Bei (*5*) liegt das nicht radioaktive Alanin.

Ergebnisse. Wird markiertes Glykokoll jungen, intensiv wachsenden Pilzkulturen (in den ersten 6 Tagen nach Beimpfung der Kolben) verabreicht, erscheinen in den folgenden 24 h rund 2% der Radioaktivität im Kohlendioxyd. Die Bildung von markiertem Kohlendioxyd ist in den ersten Stunden nach Zugabe am stärksten und nimmt nachher rasch ab; die 2% entsprechen deshalb praktisch der gesamten im Kohlendioxyd abgegebenen Radioaktivität. Das von älteren, weniger rasch wachsenden Kulturen abgegebene Kohlendioxyd ist nur in Spuren radioaktiv. 4 h nach Glykokollzugabe findet sich der grösste Teil der Radioaktivität des Proteinhydrolysates im Glykokoll, Serin, Valin und Leucin; Asparaginsäure und Glutaminsäure sind nur wenig radioaktiv. Serin, Asparaginsäure und Glutaminsäure wurden als bekannte Umwandlungsprodukte des Glykokolls bereits erwähnt; dagegen sind *Valin* und *Leucin* unseres Wissens bisher noch nicht nachgewiesen worden. Über den Mechanismus dieser Reaktionen kann zur Zeit noch nichts ausgesagt werden.

Der *Fritz-Hoffmann-La-Roche-Stiftung* zur Förderung wissenschaftlicher Arbeitsgemeinschaften in der Schweiz, der Schweizerischen Koordinationskommission für die technische Hilfe und der *Volkart-Stiftung* möchten wir für die Ermöglichung dieser Untersuchungen auch an dieser Stelle bestens danken.

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und D. KLUEPFEL

Institut für spezielle Botanik der Eidgenössischen Technischen Hochschule, Zürich, den 5. April 1957.

Summary

C^{14}-methylene-labelled glycine was added to growing mycelium of *Fusarium lycopersici*. Young, rapidly growing mycelium converts about 2% of the radioactivity into carbon dioxyde; with older mycelium, the carbon dioxyde is almost inactive. In the protein hydrolysate, labelled glycine, serine, valine and leucine and small quantities of aspartic acid and glutamic acid were found. Valine and leucine apparently have not been identified before as metabolic products of glycine.

[7] B. D. SANWAL, in: *Papierchromatographie in der Botanik* (Springer, Berlin, Göttingen und Heidelberg 1955), S. 30. – H. DÖRFEL, l.c. 105.

Effect of Mutagens on the Content of Nucleic Acids in Wheat

It has been noticed by HEVESY[1], SKIPPER and MITCHELL[2], HOLMES[3] and many others that irradiation with a considerable range of X-ray doses reduces the rate of deoxyribonucleic acid (DNA) formation in growing tissues. Inhibition of ribonucleic acid (RNA) synthesis has been found to be less marked, or synthesis may even be increased. These results are mostly based on experiments carried out in animal tissues or in microorganisms; studies in plant material have been comparatively few. SISSAKIAN[4] has recently reported the effects of X-ray radiation on the biosynthesis of nucleic acids in rye seedlings. He found that the treatment affected adversely the DNA metabolism and that DNA was more susceptible than RNA to radiation damage. The present study was undertaken (1) to compare the effects of treatment with physical and chemical mutagens on the synthesis of nucleic acids in wheat seedlings and (2) to estimate the relative effects of X-ray radiation on DNA and RNA synthesis at different stages in the post-irradiation development of treated seedlings.

For a comparative study of the effects of different mutagens on nucleic acid synthesis, dormant seeds of *Triticum aestivum* (var. N.P. 718) were treated with X-rays (50 KV; unfiltered), mustard oil from *Brassica campestris* var. *toria* and nitrogen mustard (Di 2-chloroethyl-methylamine hydrochloride). The dosages used were 11000 r of X-rays, 0·0005% nitrogen mustard for 40 min and mustard oil for 6 h. Mustard oil was included in the study in view of its capacity to induce chromosome breakage[5]. Estimations of DNA and RNA contents were carried out in 3-day-old seedlings by the method of OGUR and ROSEN[6] and submitted to phosphorus analysis. The plumule and radicle were separately analysed and the experiment was replicated thrice. The mean values of DNA and RNA content found per unit weight of radicle and plumule are given in Table I. It will be seen that there is a decrease in the content of both DNA and RNA in the radicles and plumules of treated seedlings and the percentage of reduction in each treatment is given in Table II. In the case of X-ray and mustard oil treatment, the plumule showed a greater percentage of reduction than the radicle. All the differences are statistically significant.

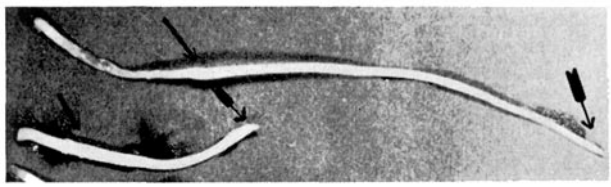
There was complete inhibition of growth of both plumule and radicle after 72 h in seedlings from the nitrogen mustard treatment and this may account for the uniform reduction in the DNA and RNA contents in the two tissues. X-rays and mustard oil, however, caused a greater inhibition of the shoot part of the plant. In the root meristems, there was a tendency for a rapid elimination of aberrant cells. This was particularly evident in *Allium* root tips treated with mustard oil, where the meristems which developed subsequent to the treatment had practically all normal cells, even though a large percentage of cells showed aberrations in the region of the tumour which developed soon after the treatment (Figure). Some such factor may be responsible for the reduced susceptibility observed of the radicle to

[1] G. HEVESY, Nature *163*, 869 (1949).
[2] H. E. SKIPPER and J. H. MITCHELL, Cancer *4*, 363 (1951).
[3] B. E. HOLMES, Brit. J. Radiol. N. S. *22*, 487 (1949).
[4] N. M. SISSAKIAN, Proc. International Conference on Peaceful uses of Atomic Energy *11*, 248 (1956).
[5] M. S. SWAMINATHAN and A. T. NATARAJAN, Curr. Sci. *25*, 382 (1956).
[6] M. OGUR and G. ROSEN, Arch. Biochem. *25*, 262 (1950).

Table I
Effect of mutagens on the content of Nucleic Acids in 3-day-old wheat seedlings.

Treatment	DNAP in $\mu g \pm$ S.E		RNAP in $\mu g \pm$ S.E		DNAP/RNAP	
	Radicle	Plumule	Radicle	Plumule	Radicle	Plumule
Control	55·22 ± 0·71	46·68 ± 0·53	64·10 ± 0·92	39·27 ± 0·69	0·8614	1·188
X-rays	51·56 ± 0·72	41·16 ± 0·96	56·40 ± 0·89	31·50 ± 0·58	0·914	1·306
Mustard oil	52·80 ± 0·59	33·23 ± 0·67	54·46 ± 0·97	29·76 ± 0·51	0·969	1·116
Nitrogen Mustrad .	44·66 ± 0·60	37·16 ± 0·50	43·30 ± 0·65	26·13 ± 0·94	1·031	1·422

the action of X-rays and mustard oil. Also, the higher DNA/RNA ratio found in the plumule may contribute towards its greater sensitivity. Quantitatively, the trend of action of X-ray and chemical mutagens on the nucleic acid synthesis appears to be fairly similar, although the difference in the sensitivity between radicle and plumule is more pronounced in the mustard oil treatment. Although it is difficult to equate the action of X-rays and chemicals on chromosomes and other cell constituents, it is probable that through different pathways of action a comparable end-result is obtained.



Onion roots 3 days after treatment with mustard oil for 2 h. The meristem in contact with the oil gets swollen (→) and consists of many cells with nuclear aberrations. The meristem developed subsequently from the tumour region (→) consists of mostly normal cells.

Besides a decrease in the content of both DNA and RNA in all the treatments, the data in Tables I and II show a greater susceptibility of RNA than DNA to the action of the mutagens. These results are contrary to those reported by TRUDOVA[7] in l. c. and SISSAKIAN[4] in rye, who found that DNA is more sensitive than RNA to the action of X-rays. These authors, however, have

Table II
Percentage of reduction caused by mutagens in the content of Nucleic acids.

Treatment	DNAP		RNAP	
	Radicle	Plumule	Radicle	Plumule
X-rays	6·7	11·9	12·10	19·8
Mustard oil	4·4	28·9	8·48	24·2
Nitrogen Mustard	19·2	20·4	32·50	33·5

used seedlings for treatment and have also carried out the estimations soon after irradiation. Since, in our first experiment, dormant seeds were used for the treatment and the content of nucleic acid was measured in 3 day-old seedlings, another experiment was performed in which 2 day-old seedlings were irradiated with 5000 r of X-rays and the DNA and RNA contents were measured separately in the radicles and the plumules 2, 24, 72, and 96 h after treatment. Measurements were also carried out at each stage in control seedlings of comparable ages In dormant seeds irradiated with 11 000 r of X-rays, as

Table III
Effect of treatment of dormant wheat seeds with X-rays (11000 r) on the content of Nucleic acids in 1-day- and 3-day-old seedlings.

Stage of Analysis	DNAP in μg	RNAP in μg
24 h after germination: Control . .	61·38	60·99
Treated. .	42·98	53·76
72 h after germination: Control . .	50·95	51·69
Treated. .	46·36	43·95

in the first experiment, estimations were done 24 and 72 h after the germination of seeds. The data obtained are summarized in Tables III and IV. The percentage of

Table IV
Effect of treatment of 2-day-old seedlings with X-rays (5000 r) on the content of Nucleic acids at different post-irradiation stages of development

Stage of Analysis	DNAP in μg		RNAP in μg	
	Plumule	Radicle	Plumule	Radicle
2 h after irradiation: Control	48·18	57·49	42·81	63·84
Treated	43·26	54·84	39·92	63·78
24 h after irradiation: Control	46·91	55·30	38·70	64·30
Treated	31·68	39·99	32·83	60·40
72 h after irradiation: Control	47·90	58·20	41·62	65·30
Treated	36·71	45·95	34·25	59·20
96 h after irradiation: Control	47·63	59·00	41·35	65·60
Treated	43·21	53·67	35·23	60·10

reduction in DNA and RNA content at different post-irradiation stages of development is given in Table V.

Table V
Percentage reduction in the content of Nucleic acids at different post-irradiation stages in wheat treated with X-rays.

Treatment	Stage of Analysis h	DNAP	RNAP
Seed treatment (11 000 r)	24	29·9	11·8
Seed treatment (11 000 r)	72	9·3	15·9
Seedling treatment (5000 r)	2	7·4	3·4
Seedling treatment (5000 r)	24	30·0	10·6
Seedling treatment (5000 r)	72	22·2	14·0
Seedling treatment (5000 r)	96	9·1	11·5

[7] R. G. TRUDOVA, cited by N. M. SISSAKIAN ([4]).

From the data, it appears that soon after irradiation there is a greater reduction in DNA content, in comparison with RNA, as reported by previous workers. Subsequently, however, the trend is reversed, there being a greater percentage of reduction in RNA than DNA content. (Table V; 3 days after seed treatment and 4 days after seedling treatment.) Thus, it seems likely that DNA synthesis, although initially it may be more affected than RNA, returns to normalcy at a more rapid rate than RNA following treatment with mutagens at a sub-lethal dosage. It is hence important to take into account the developmental stage at which the estimations are carried out in studies of this nature.

We are indebted to Dr. B. P. PAL and Dr. S. M. SIKKA for their interest in the study and encouragement.

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Indian Agricultural Research Institute, New Delhi (India), February 18, 1957.

Zusammenfassung

Eine vergleichende Untersuchung über die Wirkung von Röntgenstrahlen auf ruhende Weizensamen und Weizenkeimlinge und von Senföl auf den Gehalt von DNA. und RNA. in Spross und Wurzel von Keimlingen verschiedenen Alters wurde ausgeführt.

Fusaric Acid Production by *Fusarium orthoceras in vitro*

Fusaric acid, a wilt toxin produced by certain species of *Fusarium*, is known to be synthesized in culture medium by *Fusarium heterosporum* Nees[1], *F. bulbigenum* Cke. et Mass. var. *lycopersici* (Brushi) Wr. et Rg., *F. vasinfectum* Atk., *Gibberella fujikuroi* (Saw.) Wr.[2], and *Nectria cinnabarina* (Tode) Fr.[3] The detection of this toxin *in vivo* in *F. vasinfectum* infected cotton plants has also been reported[4]. Recently, in an extensive study of 23 species of *Fusarium* (obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland) it was observed that in addition to the species already reported, *F. orthoceras* App. et Wr. was capable of producing appreciable quantities of fusaric acid in culture.

Spore suspension of *F. orthoceras* was inoculated in 50 ml portions of Richard's medium in 250 ml conical flasks and incubated at laboratory room temperature (27–29°C). After 3 weeks, the fungal mat was filtered and the filtrate tested quantitatively for fusaric acid by bioassay[5]. The identity of the toxin in the filtrate was established by chromatographic determination of its R_f value in known solvents[6]. Approximately 50 ml of the filtrate were reduced to 1 ml volume *in vacuo* and the concentrated filtrate was spotted on filter paper strips and run in butanol-acetic acid-water (4:1:5) in test-tubes[7]. Culture filtrate of *F. moniliforme*, known to contain fusaric acid, and dilute solutions of pure fusaric acid were similarly spotted and run. The paper strips were air dried and spread over bacterial seeded agar[8] and incubated at 37°C for 18 h, when a clear zone of inhibition was formed around the position occupied by the toxin and pure fusaric acid on the filter paper. The R_f value of pure fusaric acid was found to be 0·89 and that of the toxin present in the filtrates of *F. orthoceras* and *F. moniliforme* was 0·87. R_f value of fusaric acid, when added to culture filtrate and similarly spotted and run in the solvent, was found to be 0·87. The lower R_f value of the toxin, when present in the filtrate, was probably due to salts and other interfering substances in the filtrate.

Under identical conditions, a three-weeks-old culture of *F. orthoceras*, grown in 50 ml Richard's medium in 250 ml conical flasks, produced 300 mg/l fusaric acid, whereas *F. moniliforme* and *F. vasinfectum* produced only 65 mg/l and 35 mg/l fusaric acid, respectively.

Dialyzed culture filtrate of *F. orthoceras* at 5 and 10% concentrations incited typical vein clearing symptoms[2] in cut shoots of susceptible cotton (Karunganni 2-*Gossypium arboreum*), thus further confirming the presence of fusaric acid.

I am much indebted to Prof. T. S. SADASIVAN for guidance and to Dr. E. GÄUMANN for the sample of fusaric acid. I thank Dr. C. V. SUBRAMANIAN for his interest in this work and the National Institute of Sciences of India for the award of an I.C.I. Research Fellowship.

C. S. VENKATA RAM

University Botany Laboratory, Madras, India, June 28, 1956.

Zusammenfassung

Durch biologische und papierchromatographische Methoden konnte nachgewiesen werden, dass auch *Fusarium orthoceras* den Welkestoff Fusarinsäure in die Kulturflüssigkeit abscheidet. Unter den angegebenen Bedingungen werden relativ grosse Mengen dieses Stoffes gebildet (300 mg/l gegenüber 65 mg/l durch *F. moniliforme* und 35 mg/l durch *F. vasinfectum*).

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[2] E. GÄUMANN, S. NAEF-ROTH, and H. KOBEL, Phytopath. Z. *20*, 1 (1952).
[3] E. GÄUMANN, Endeavour *13*, 198 (1954).
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[8] H. ZÄHNER, Phytopath. Z. *22*, 227 (1954).

Untersuchungen über den Ascorbigengehalt von Kohlrabi (*Brassica oleracea* v. *gongylodes*) während der Vegetation und den Zusammenhang zwischen Ascorbigen und Wachstum bei den Pflanzen der Familie Brassicacae

Ascorbigen kommt in den Pflanzen der Familie Brassicacae vor und ist eine oxydationsbeständige Verbindung der Ascorbinsäure mit einem Indolderivat (Indolylpropendiol)[1]. Über seine physiologische Bedeutung für die Pflanze war bisher nichts bekannt. Als Beitrag zur Aufklärung dieser Frage verfolgten wir in unserer Arbeit

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