ceptors of physalaemin exist on the TAN neuromembrane, and that the majority of the inhibitory receptors is situated more superficially than the excitatory ones. The excitatory effect of physalaemin is clearly predominant to the inhibitory effect. We believe that the excitatory effect masks the inhibitory one, when physalaemin penetrates into the depth of the ganglia and acts on the TAN excitatory receptors. Hence, during bath application of this peptide, we notice only an excitatory effect.

ERSPAMER et al.[3] reported that physalaemin greatly lowered the blood pressure of some mammals when injected i.v. and that this peptide stimulated directly some mammalian smooth muscles (large intestine and ileum), just like eledoisin[6], a peptide extracted from the posterior salivary glands of eledone.

On the central nervous system, KONISHI and OTSUKA[7] reported that a change of the ventral root potential (a mass response of neurones) of the bullfrog spinal cord was similarly caused by the administration of each of the 3 hypotensive peptides, substance P, physalaemin and eledoisin. They assumed that the common C-terminal sequence of these three peptides remarkably excited spinal motoneurones.

We demonstrated in the present study that physalaemin had effects on the excitability of a molluscan giant neurone, the TAN, as well as mammalian smooth muscles or amphibian spinal cord neurones. Of the vaso-active peptides examined, however, the TAN neuromembrane was selectively sensitive to physalaemine, unlike the mass response of the amphibian spinal cord neurones observed by KONISHI and OTSUKA.

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Toxic Substances Produced by *Fusarium*. III. Production and Screening of Phytotoxic Substances of *F. oxysporum* f. sp. *carthami* Responsible for the Wilt Disease of Safflower *Carthamus tinctorius* Linn.

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Summary. Fusarium oxysporum f. sp. *carthami*, a causative agent for the wilt disease of safflower (*Carthamus tinctorius* Linn.), has been shown to produce diacetoxyscirpenol, T-2 toxin, fusaric acid and lycomarasmin in artificial media. These substances produced disease syndromes, similar to those seen after the natural infection, when administered in healthy plants. Diacetoxyscirpenol and T-2 toxin have been detected in diseased safflower plants after inoculating with the wilt pathogen. This study is the first demonstration of vivotoxicity of diacetoxyscirpenol.

Safflower (*Carthamus tinctorius* Linn.) is an important oil seed crop cultivated in several States of India. The seeds are edible and are used in culinary purposes; the oil cake is used as a cattle feed. Two new diseases, viz., wilt and dumping off, of safflower, surveyed in the Varanasi and Mirzapur Districts of India, were reported recently[2]. The causative agent for the wilt disease was found here[2] and elsewhere[3] to be *Fusarium oxysporum* f. sp. *carthami*. The nature of substance or substances responsible for the phytotoxic effects has not been evaluated until this investigation. Since food materials infected with fusaria have often been found to contain substances which produce high mammalian toxicity[4], the presence of the title *Fusarium* species in safflower is also important from the public health aspect. The present investigation was designed to isolate and study the phytotoxic substances, produced by the fungus in artificial media and in vivo, and their adverse effects on the host tissues.

The fungus was collected from Varanasi and its identity (IMI-166917) was confirmed by the Commonwealth Mycological Institute, Kew, England. It was grown in Richards solution (200 ml) in still culture flasks (1 l) at 21 °C for 21 days. In a preliminary screening, the effect of the culture filtrate on safflower seedlings was determined. The usual toxic symptoms produced by the natural infection were manifested after administration of the culture filtrate. The phytotoxic substances were extracted from a larger volume of the culture filtrate (5 l) by successive extractions with chloroform (3 l), ethyl acetate (3 l) and *n*-butanol (2 l). The residue from the chloroform extract, containing several trichothecene derivatives[5], produced, in high dilutions, toxic symptoms on safflower seedlings some of which were similar to those showed by the culture filtrate. Clearly, therefore, some more constituents in the culture filtrate are responsible for the total toxic symptoms. The search for these constituents in the ethyl acetate and *n*-butanol extracts resulted in the isolation of fusaric acid and lycomarasmin from these extracts. Additionally, several unidentified nitrogenous components were detected in the latter two extracts. The residue from the EtOAc extract, a pale brown amorphous solid (1.2 g), showed 3 major ninhydrin-positive spots on TLC (silica gel G, E. Merck, thickness, 0.4 mm) and PPC (Whatman No. 1) using *n*-BuOH-AcOH-H_2O (4:1:2) as the developer. The residue was triturated with hot hexane and the hexane-soluble part was filtered. The filtrate was set aside giving straw-coloured crystals (112 mg), m.p. 101–102°; UV: λ_{max} (EtOH) 230 (log ε, 4.02), 272 nm (log ε, 3.64); MS: *m/e* 179 (M^+), significant fragment ion peaks at *m/e* 135, 134, 122. These properties are indistinguishable from those reported for fusaric acid[6]. Processing of the *n*-BuOH extract afforded a brown gum (0.8 g) which also showed a number of prominent and diffused ninhydrin-positive spots on TLC and PPC. The residue

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Effects of secondary metabolites[a] of *F. oxysporum* f. sp. *carthami* on safflower seedlings

Symptoms due to natural infection recorded at: a) Cotyledonary stage upto first leaf b) 2 weeks after manifestation of infection	Similar symptoms shown by the 3 extractives: $CHCl_3$ (1), EtOAc (2), *n*-BuOH (3) and the culture filtrate (4)[b]
a) Yellowish discolouration at the collar region which later turns brown	4
Blackening of tap roots	2, 3, 4
Shrievelling and crinkling of leaves	3, 4
Bending of leaf lamina on midrib	1, 2, 4
Rolling of leaves	3, 4
Brown necrotic dots on leaves	1, 2, 4
b) Chlorosis	1, 2
Scorching of lamina (necrotic patches)	1
Epinasty	2
Bending of leaf lamina on midrib	1, 2
Flacidity	1, 2
Browning of vascular strands	1

[a] At both concentrations (high and low) similar symptoms appeared; in case of low concentration, the onset of action was delayed. [b] Effect of the culture filtrate was determined only on cotyledonary seedlings.

was dissolved in methanol and the solution was concentrated when lycomarasmin separated as microcrystals (66 mg), m.p. 225–228° (lit.[7] m.p. 227–229°). Hydrolysis of this compound with 1 *N* aq. HCl, on a steam bath for 6 h furnished glycine and aspartic acid.

Assays were conducted with the 3 extractives at ordinary temperature and humidity. Two concentrations of each extractive ($CHCl_3$ extractive, 0.028 µg/ml and 0.28 µg/ml; EtOAc, 0.88 µg/ml, 8.8 µg/ml; *n*-BuOH, 0.52 µg/ml, 5.2 µg/ml) were prepared using Hoagland's solution for dilution. Each of these solutions (20 ml) was taken in a culture tube wrapped with black paper into which 1 disease-free seedling of safflower was introduced. Only the roots were kept immersed in solution. Hoagland's solution was used as control. In another experiment, each of the above solutions (1 ml) was injected to the plant system at the collar region. The results are recorded in the Table. It would seem from the results that the phytotoxic activity of the fungus is not due to a single entity but due to additive effects of the secondary metabolites produced in vitro.

The fact that the fungus is capable of producing highly toxic substances under not very critical conditions, carries with it the possibility that the substances can be produced in the host tissues after the infection. It has been demonstrated here for the first time that trichothecenes, which are known to cause high mammalian toxicity[4], can be translocated in the host tissues from the roots. This study has also established, dispelling earlier doubts, that diacetoxyscirpenol is indeed a vivotoxin. In order to prove this, safflower plants were grown on sterilized soil infected with the fungus. After 60 days, when distinct disease symptoms appeared on the leaf, stem and roots, the plants were harvested. The individual parts were washed and then macerated with water and chloroform in a high speed blender. The chloroform extracts were processed in the usual way. The residue showed the presence of diacetoxyscirpenol and T-2 toxin by its fluorescence under UV-light (short wavelength) on TLC, development of purple and violet colours with Ehrlich reagent, and by the capacity to kill apical buds of safflower which was followed by the appearance of new shoots of auxilliary buds. This last effect is very similar to that reported[8] for diacetoxyscirpenol on winter tares. The amounts of the trichothecene derivatives were maximum in the roots and minimum in the leaves. The translocation of the trichothecene derivatives was demonstrated by their movement through conductive tissues when the total chloroform extractive was injected or when it was soaked in the root system. On one occasion, even when the mycelium was absent, diacetoxyscirpenol was isolated from seeds of safflower.

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β-Blockade of Morphine-Induced Hyperlactacidemia in Rabbits[1]

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Summary. In morphinized rabbits blood lactate levels are elevated. Hyperlactacidemia persists after cessation of morphine injections. This morphine-induced lactate accumulation is completely abolished by simultaneous propranolol treatment. Phentolamine does not modify the action of morphine.

In morphinized animals, blood and various other tissues have elevated lactate levels[3,4]. This indicates a profound change in cellular metabolism which might be related to the characteristic disturbances of the abstinence syndrome. The mechanism responsible for the hyperlactacidemia following morphine administration is not yet clear. It is possible that it results partially from catecholamine secretion, at least for the first few injections. We have tried to prevent the lactate accumulation which occurs in morphinized rabbits by pretreating the animals with adrenoblocking agents: phentolamine and propranolol.

Materials and methods. Male Fauve de Bourgogne rabbits weighing 2.5 kg were used. Blood samples were taken from the marginal vein of the ear at 09.00 h in 15 h-fasted animals. Morphine hydrochloride (Chaix and du

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