sodium carbonate (2%) solution, were uniformly sprayed over the inoculated areas. In another set of experiments, the order of spraying of the fungal spores and of the test compounds was reversed. In the control, similar number of ears was sprayed only with the fungal spores. The cobs were then covered with polythene bags. The treated and the control ears were harvested after a 2-week period. For each experiment, 10 replicates were maintained.

To determine the effect of the 3 test compounds on the fungal growth in vitro, each of the test compounds (1×10^{-4} M) was taken in sterilized Richard's solution (50 ml in a 250 ml Erlenmeyer flask) to which the fungus was inoculated. The mixture was incubated (temperature 18 °C) for 7 days. The fungal mat was collected by filtration and its dry weight was noted. For each test compound, 5 replicates were maintained.

Results and discussion. The ears inoculated with the fungal spores showed the disease symptoms (table 1), within 72 h, similar to those observed in the natural infection. In the control, the pathogen first damaged the kernels at the tip and then proceeded downward and into the layers of the husks. Pretreatment of the ears with 2,2',4-trihydroxybenzophenone provided significant protection from the ingress of the fungal hyphae. Also, considerable recovery of the host from the fungal infection was observed when the benzophenone was sprayed 3 days after the spray of the fungal spores. Pretreatment of the ears with mangiferin also provided some degree of protection from the fungus invasion as was revealed from the considerably diminished number of infected kernels and from the normal formation of the grains. However, mangiferin did not produce any curative action of the infected grains or husks. The potency of antifungal action of 1,3,6,7-tetrahydroxyxanthone was of intermediate order of the other 2 test compounds (table 1).

In vitro experiments with the 3 test compounds resulted, in each case, in the lysis of hyphal cells and considerably reduced growth of the fungus. The results are recorded in table 2.

In view of the well-documented monoamine oxidase inhibitor activity of mangiferin[6], it would seem likely that this could be the mode of the antifungal action of the 3 test compounds against *F. moniliforme* Sheld. This study has assumed additional significance since toxicity of natural products from *Fusarium* has received wide attention in recent years[7-9] because of the greater incidence of the fusarial toxins and perhaps of their greater importance than aflatoxins[10].

Table 2. Effect of polyphenolic compounds on the growth of mycelium of *Fusarium moniliforme* Sheld

Test compound	Dry weight and nature of fungal mat
Mangiferin	0.348 g; lysis of the mycelia, the mat became black, the protoplast of the hyphae became contracted and collected in the centre or at the corner of the hyphal cells.
1,3,6,7-tetrahydroxy-xanthone	0.335 g; appreciable blackening of the mat and lysis of the hyphae.
2,2',4-trihydroxy-benzophenone	0.10 g; no mycelial growth upto 72 h, subsequently only feeble growth followed by lysis of the hyphae.
Control	0.90 g; normal light pink coloured mycelia.

* Part 9 in the series 'Toxic substances produced by *Fusarium*'. For part 8 see S. Ghosal, K. Biswas and B.K. Chattopadhyay, Phytochemistry *17*, 689 (1978).

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Studies of the mechanism of action of the carotenoid crocetin

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Summary. The carotenoid crocetin was found to be taken up by normal rat muscle cells grown in vitro, and was found in the ribosomal-microsomal fraction, supporting the hypothesis that crocetin affects cell division enzymatic processes.

Crocetin is a carotenoid compound which appears to enhance oxygen diffusivity in vitro[2,3] and in vivo[4,5]. Based on several theories which support the hypothesis that deficiency of oxygen (hypoxia) is the initiator of diseases[6], the application of crocetin to several of these diseases has shown to be effective[7,8]. An example is the radio-sensitization of Walker 256 carcinoma in vivo as well as in vitro[9]. As tumor cells are known to be hypoxic and therefore radio resistant, by administering crocetin the tumor could be cured using lower radiation dosages[9,10]. Oxygen therapy also results in tumor cures at lower radiation dosages. Further, crocetin lowered the cholesterol and triglyceride level of plasma, and the severity of atherosclerotic lesions in the aorta of high-cholesterol diet fed rabbits[5,6]. The action of crocetin in this case may be due to 2 effects: 1stly, increasing oxygen transport and therefore decreasing the severity of the initiation of the sclerotic region, and 2ndly, presumably decreasing the cholesterol adsorption from the gastrointestinal tract. Increased oxygen environments have been shown previously to have the same effects on atherosclerosis in rabbits[5,6].

Crocetin is a carotenoid as is vitamin A, the precurser of ocular rhodopsin. Using rats as experimental animals, it has been found that the amount of ocular rhodopsin in the

crocetin-injected groups is double the amount in the control animals (Gainer and McKinnon, personal communication).

This paper reports work on the cellular and molecular mechanism of action of crocetin, using cell culture and cell fractionation methods. It was necessary to develop an assay for crocetin (to be described elsewhere), fluorescence and spectrophotometer techniques being investigated with the latter proving most useful.

Material and methods. Western albino young rats were utilized. Muscle pieces were removed aseptically after anesthetizing the rat with urethane. The mass of muscle was transferred to a petri dish containing Hanks balanced salt solution (BSS) with antibiotics (Na penicillin, streptomycin sulphate, and a fungicide). The muscle was quickly cut into very small pieces. Some of these fragments were trypsinized with 0.25% trypsin, centrifuged and suspended in media[11]. The media utilized was Eagles modified media supplemented with 10% calf serum and the same antibiotics (Grand Island Biological Company, Grand Island, N.Y.). The culture flask was a Falcon of 30 ml capacity. The air of the flasks was replaced by a gas mixture of 5% CO_2 and 95% air, and they were incubated at 37 °C. Healthy cells appeared and reached confluency within 3 weeks. For cell propagation, standard procedure for trypsinization and reinoculation was followed[11]. Before reinoculation the cells were counted utilizing a hemocytometer and trypan blue for viability; each flask contained 10^4–10^6 cells/whole flask. Figure 1 describes the spectrum of the crocetin solution used throughout the present in vitro studies. 1 mg/ml crocetin solution of pH ~ 8 was prepared by dissolving 1 g of crocetin in 50 ml 2 N NaOH, then adding 50 ml 2 N HKl until the pH was 8, then diluting 1:10. The spectrum has 3 peaks in the visible, IR-, and UV-regions. The maximum peak is at 423 nm.

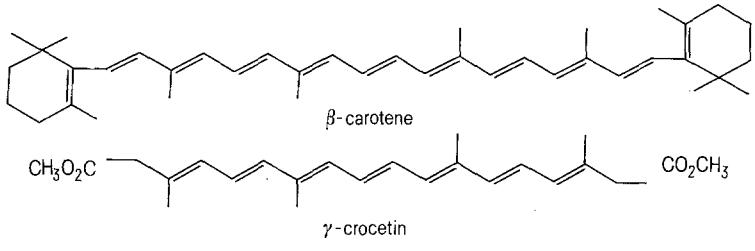
For kinetic measurements, the concentration change with time after the flasks reached confluency was used. The cells were washed with Hanks (BBS) as the phenol red in the media interfered with spectrophotometric measurements, and sampling was after addition of 1 ml of 0.1 mg/ml crocetin solution to 4 ml of Hanks and 1 ml of plasma. The time period for sampling differed according to whether the previous reading changed reasonably, but ranged from 2 h to 5 h, for a total of 11 h.

The cells were washed with Hanks solution thoroughly, and the last wash was checked for the absence of crocetin. The cells were then trypsinized and resuspended in Hanks, and all the cells pooled into one flask containing 10 ml of Hanks. Using a glass homogenizer the cells were disrupted mechanically. The resulting homogenate consisted of a heterogenous suspension of unbroken cells and different organelles of the cells in Hanks BSS. Successive centrifugation and separation of the sediment for each organelle was carried out using standard procedures[12,13].

Final separation was at 40,000 × g and no further separation could be made. Crocetin was identified in certain organelles. The sediments were resuspended in Hanks, and these suspensions and the last supernatant were scanned with a spectrophotometer.

Crocetin, a conjugated, dibasic, hydrocarbon acid, is one of the family of carotenoid compounds. Although certain analogous carotenoids have a similar ability to raise diffusivity of gases in aqueous solution, crocetin and its salts are by large measure the most effective[8].

The structural relationship of crocetin to β-carotene[14] is clear:



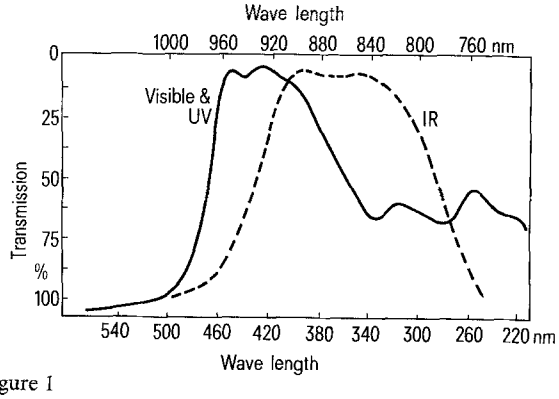


Figure 1

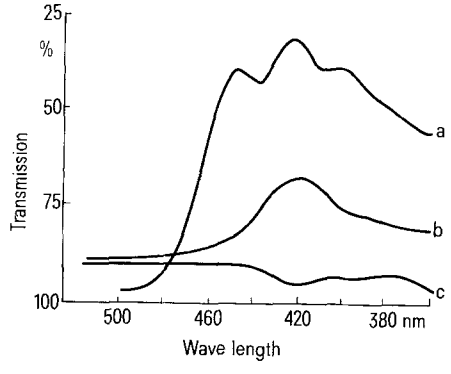


Figure 3

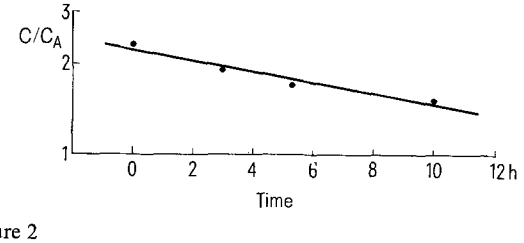


Figure 2

Fig. 1. Transmission spectrum of 0.1 mg/ml diluted 1:10 crocetin in 2N-NaOH neutralized with 2N-HCl for pH = 7.

Fig. 2. Reaction rate kinetics of crocetin with derived normal rat muscle cells at 425 nm.

Fig. 3. Scanning transmission spectra of: *a* Crocetin in Hank (BSS); *b* ribosomal-microsomal fractions of the cells treated with crocetin; *c* ribosomal-microsomal fractions of the control cells.

According to Karrer et al.[15-18], crocetin has 3 analogues, and the α, β forms hydrolyze to γ crocetin in 10% NaOH or KOH. The α form in 10% NaOH, pH adjusted to ~ 8 by HCl, has been used throughout the present experiments.

Bearing in mind that receptors for most drugs have yet to be identified, there is little doubt that drug-cell combinations obeying mass law kinetics are involved in drug action. Therefore an approach based on kinetics can be expected to yield useful results in determination of the reaction mechanisms. Figure 2 shows a plot of log C/C_A vs time where

C = the concentration of crocetin at time t, C_A = the initial concentration.

It is seen from figure 2, a straight line relationship is obtained, therefore indicating that the crocetin uptake by the cells is first order, with a rate constant of 0.46 sec^{-1}. The rate is neither very fast nor very slow, so the process is neither diffusion nor reaction controlled[19,20].

The sediments after $1000 \times g$ (10 min), $10,000 \times g$ (30 min), and $40,000 \times g$ (2 h) were suspended in BSS. The remaining supernatant after $40,000 \times g$ and each resuspended sediment were assayed for the presence of crocetin using the spectrophotometric method developed. A very distinctive crocetin peak was identified in the microsomal-ribosomal cell fraction, figure 3.

The finding is not surprising, as crocetin enhances cell growth, and is thought to be involved in the enzymatic processes which regulate cell division[21]. Also it is possible that it might have a role in the electron transport chain, since it apparently increases oxygen transport. Further studies of microsomal-ribosomal fractions should be conducted separately.

Conclusion. Crocetin and similar compounds have great potential for treatment for several diseases, especially those in which hypoxia is believed to be a significant causal factor. This is due to the action of crocetin in increasing oxygen transport in plasma, tissues, and in cells. Elucidation of the precise mechanism of action of crocetin is needed so more potent similar compounds can be obtained. Moreover, therapeutic uses of crocetin can be used most effectively by knowing its mechanism of action.

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Inhibition of protein synthesis in ischaemic liver from phenobarbitone-treated rat

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Summary. Both ribosomal factors and cytosolic inhibitors are involved in the reduction of the rate of protein synthesis which occurs in the ischaemic hepatocyte from control and phenobarbitone-treated livers. Of these 2 factors it is the latter which seems to play a major role in determining the irreversible impairment of protein synthesis. Phenobarbitone administration has no effect on the rate of protein synthesis of ischaemic and post-ischaemic hepatocyte.

Liver ischaemia entails a reduced capacity for protein synthesis which becomes irreversibly impaired if the insult lasts longer than 60 min[2]. This results from disaggregation of the polyribosomes and modifications of the cell sap[3,4]. Accordingly, we set out to determine, in reversible and irreversible liver ischaemia, a) the relative contribution of ribosomal activity and cytosolic factors to the inhibition of protein synthesis, and b) if treatment with phenobarbitone, a substance known to increase the rate of protein synthesis in vivo[5] and in vitro[6,7], could favorably influence the capacity for recovery of the hepatocyte.

The results obtained indicate that a) though both ribosomal factors and cytosolic inhibitors are involved in the impairement of protein synthesis in the ischaemic hepatocyte, it is the onset of the latter which seems to determine the irreversibility of the ischaemic lesion, and b) that phenobarbitone administration has no effect on the capacity of the hepatocyte to withstand the ischaemic insult.

Materials and methods. Chemicals: These were as previously specified[7]. Animals: Male albino rats (Wistar strain) fed on a diet of laboratory chow (Piccioni, Brescia, Italy) were starved for 14 h before the experiment. Phenobarbitone