peared in all the seedlings of the control group. In case of the benzophenone-treated group, healthy seedlings emerged and no disease symptoms appeared, also thereafter.

The effect of the benzophenone on the fungal hyphae was examined. The fungus was grown (48 h) in sterilized Richard's medium (150 ml) to which the benzophenone was added in 3 different concentrations. The extent of the mycelial growth in the control and benzophenone-treated groups was quantitated as follows. The mixtures were incubated at 21 °C for 7 days. Subsequently, the dry weight of the mat grown in the control and the benzophenone-treated groups was recorded. Lysis of the hyphal cells was observed within 72 h of the benzophenone treatment, the mycelium became black, and protoplasts were disintegrated. Shrinkage of the cell well was followed by the dissolution of the cell wall in some of the hyphae. The major portions of the mycelium was not stained when lactophenol cotton blue was applied. At the lowest concentration (1×10^{-5} M), however, the benzophenone appeared to promote growth of the mycelium, while at the highest concentration (1×10^{-3} M), it significantly retarded the mycelial growth. The mycelial growth (in g $\pm$ SEM) in the control and the benzophenone-treated (1×10^{-3} M) groups were 0.422 ± 0.0023 and 0.118 ± 0.002, respectively ($p < 0.01$).

The potentiality of the benzophenone as a foliar fungicide was examined. The benzophenone was sprayed on the cotyledon leaves of 2-day-old seedlings showing typical symptoms of the infection. Remarkable recovery from the affliction was observed in the treated group. The first leaves of the treated seedlings were normal in size and shape, while those in the control group showed typical abnormality associated with the infection.

The ability of translocation of the benzophenone from leaves to roots of safflower was demonstrated. 10-day-old seedlings of safflower, grown on sterilized sand, were sprayed 4 times on the *leaves* with the benzophenone at 12-h-intervals. After 24 h from the last spray, the seedlings were uprooted, surface-sterilized, and sections of the leaves, stems, and roots were cut. These were placed on PDA plates which were seeded with the pathogen (spore suspension ca. 1,000,000/ml). The plates were incubated at 21 °C for 48 h. Around the sections of the leaves, stems and roots of the treated seedlings, a clear inhibition zone of the fungus was observed. The maximum inhibition zone was observed, as expected, around the sections of the treated leaves on which the benzophenone was sprayed. Each of the root sections also showed appreciable inhibition zone.

The above results suggest that 2,2'4-trihydroxybenzophenone could be used as a protective and a curative agent against the safflower wilt. Additionally, it possesses the unique property of translocation from leaves to roots of safflower. It has thus the potentiality for use as a foliar fungicide against this pathogen. Further studies about the practical significance of these results are currently underway.

Aflatoxin metabolism and absence of cytochrome P-450 in rat colon tissue during vitamin A malnutrition[1]

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Summary. Homogenized mucosal linings prepared from vitamin A adequate and deficient male rats were used in metabolic studies of aflatoxin B_1 (AFB_1). Cytochrome P-420 was identified in both groups which metabolized AFB_1 to 4 metabolic products in vitro. The implications of this observation are discussed in relation to colon carcinoma.

Aflatoxin B_1 is a metabolite of the toxigenic fungus *Aspergillus flavus* (Link ex Fries, U.I.81), cultures of palmsap[3] and several agricultural commodities[4] which, like most lipophilic organic compounds, are known to be metabolized by the cytochrome P-450 dependent monoxygenase system present in liver microsomes[5]. Recently, it was reported[6] that dietary vitamin A caused a decline of drug metabolism in rat liver and a lowering of cytochrome P-450. Under marginal vitamin A, aflatoxin was reported[7] to induce colon carcinoma. Since the condition may occur in man, this report could be of further biochemical and nutritional interest. Thus, it seemed to us necessary to investigate the mechanistic pattern of AFB_1 metabolism in the colon under this condition.

Materials and methods. For producing vitamin A deficiency in the experimental animals, male weanling Sprague-Dawley-derived rats (50–55 g) were fed for 45 days on a corn-based diet without the vitamin. Control animals received a diet supplemented with vitamin A (5 mg vitamin A palmitate per kg diet) for the same period, both groups receiving water ad libitum. The rats were then decapitated and their colons were dissected out and washed free of fecal materials with 0.9% saline. The mucosal linings of the colon were scraped, pooled for each group and stored at 0 °C. The pooled samples were minced in 0.2 M phosphate buffer, pH 7.4, and homogenized in the buffer in a motor-driven Potter-Elvehjem Teflon-glass homogenizer (in an iced bath) at 600 rev/min. Protein and vitamin A were determined by technique of Lowry et al.[8] and Neeld and Pearson[9] respectively. While we were attempting to characterize cytochrome P-450 from this preparation by the method of Omura and Sato[10], we encountered a pig-

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Table 1. Some basic parameters of experimental animals whose colon were used for incubating AFB_1*

	No. of animals used	Food consumption (g/day rat)	Weight gain (g)	Vitamin-A-level** Plasma	Vitamin-A-level** Liver	Cytochromes*** P-420, P-450 Colon[c]	Cytochromes*** P-420, P-450 Liver	Colon mucosal protein (mg/g colon)
Deficient	30–45	16.50 ± [a] 1.85	162 ± [a] 4	0.08 ± [a] 0.01	0.04 ± [a] 0.01	0.19 ± [x] 0.03	0.18 ± [a] 0.02	9.62 ± [a] 1.05
Vitamin-A-supplemented	31–42	17.80 ± [a] 1.55	172 ± [a] 5	13.05 ± [b] 0.52	41.50 ± [b] 2.60	0.15 ± [y] 0.03	0.20 ± [a] 0.03	8.66 ± [a] 1.30

*Values represent mean ± SEM of 6 experiments with 5–7 animals per experimental subgroup. Colons were pooled for each subgroup and 3 replicates were made per assay. [a, b] Statistical significance between dietary treatments; data which show identical superscripts are not significantly different ($pF \leqslant 0.05$). For [x] and [y] at $pF \leqslant 0.01$. **Expressed as IU/100 ml of plasma or per g wet weight of liver. ***Expressed as nmoles/mg colon mucosal or liver microsomal protein respectively. [c] Represents the material with CO. P-420 λ_{max} at 420 nm. It is uncertain whether the substance has potential cytochrome property apart from carbon monoxide binding spectrum.

ment having a CO-difference spectrum absorption maximum at 420 nm, which differs from that of cytochrome P-450, and which we have identified and characterized by means of earlier studies in this laboratory[11–13].

Metabolism of AFB_1 was determined in open 25-ml Erlenmeyer flasks at 37 °C in a Dubnoff metabolic incubator, each reaction mixture containing 0.5 ml of the homogenized colon mucosal suspension equivalent to 0.5 g colon, 1.5 ml of 400 mM phosphate buffer (pH 7.4), 1.0 ml of an NADPH-generating system containing $NADP^+$ (4 mM), glucose-6-phosphate (100 mM), Torula yeast glucose-6-phosphate dehydrogenase (4 EU/ml), 2.0 ml of 1.15% KCl and 5 µl DMSO containing 10 µg/µl AFB_1. In a separate experiment, this concentration of AFB_1 was found to be saturating for 0.3– 1.25 mg colon tissue protein per incubation mixture and with time for 8 min. Boiled preparations containing the same reaction components served as controls. Incubations were carried out for 1 h and terminated by the addition of 2 ml of a saturated solution of NaCl. The incubation mixture was then extracted with chloroform:methanol (4:1) for 10 min on a Virtis Extractomatic. The chloroform extract was passed through anhydrous sodium sulphate and evaporated to dryness under an atmosphere of nitrogen. The extracts were redissolved in 1 ml chloroform and 5 µl spotted on Adsorbosil-5 activated thin-layer plates, which were developed with water-saturated chloroform:acetone (88:12) in an unequilibrated tank. Aflatoxin B_1 and its metabolites were quantitated using standard samples of aflatoxin M_1 (AFM_1), AFB_1 and AFQ_1. Quantitation was carried out with a photovolt densitometer equipped with an Autolab System 1 computing integrator. Statistical significances were calculated, using a Wang 600-14 programmable calculator and employing Wang Statistical Program for grouped data and by the nonparametric technique of Wilcoxon and Wilcox[14].

Results and discussion. Table 1 shows that the 2 experimental groups consist of identically similar animals, except that one group was deficient in vitamin A and the other was not. Since we have isolated, characterized and quantitated cytochrome P-450 in earlier experiments[11–13], it was not difficult to identify it in this experiment, but the cytochrome could not be identified in the colon preparation under the same conditions which characterized the cytochrome in liver microsomal preparation. On the other hand, we detected a pigment which binds to carbon monoxide and which, unlike cytochrome P-450, absorbs at 420 nm λ_{max}. This material is still to be purified, requantitated and characterized. Until then we assign it provisionally 'P-420' (a pigment from colon mucosal preparation whose absorption spectrum is 420 nm when bound with carbon-monoxide). Without carbon-monoxide, the 420-nm absorption peak was not found. The concentrations of P-420 and cytochrome P-450 are shown in table 1. The results (table 2) show that there is no significant difference in the unreacted (residual) AFB_1 in both animal groups. These results also show that the mucosal epithelia tissue of the colon, despite its lack of cytochrome P-450, was capable of metabolizing AFB_1 in the 2 animal groups to AFR_0, AFQ_1 and AFM_1. The deficient animals metabolize more of AFB_1 to the component products (AFR_0, AFQ_1 and AFM_1) judging from the amount of residual aflatoxin in both reactions, though there is no significant difference in these values. There are significant differences in respect of the products AFR_0 and a component of unresolved Lowry-positive fluorescent spots on the origin. This result is related to the tissue concentration of the unidentifiable pigment (P-420) content which is significantly higher in deficient animals. Although cytochrome P-450 is easily converted to cytochrome P-420[15],

Table 2. Metabolic products of AFB_1 from colon mucosal incubations*

	Deficient	Vitamin-A-supplemented
Residual AFB_1**	27.10% ± [a] 3.20	28.20% ± [a] 2.80
AFB_1 metabolites (percent/g protein/h)		
AFR_0 (aflatoxicol)	0.34% ± 0.08[a]	0.22% ± 0.05[b]
AFQ_1	0.20% ± 0.04[a]	0.21% ± 0.02[a]
AFM_1	0.10% ± 0.02[a]	0.12% ± 0.01[a]
Origin***	1.55% ± 0.10[a]	1.20% ± 0.12[b]

*As in table 1. **Expressed as a percentage of initial AFB_1 substrate. ***Lowry-positive, fluorescent spots of AFB_1 origin.

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because of the difficulty that may be involved in solubilizing and resolving epithelial microsomes of the colon, it is impossible to determine at this stage whether this conversion (cytochrome P-450 → P-420) occurred during tissue preparation. The detailed chemistry of ligand interaction with the protoheme of cytochrome P-450 and the uncharacterized pigment (with CO P-420 λ_{max} about 420 nm) encountered in our preparation remains a challenge for future studies. Since colon carcinoma has been induced by AFB_1 in vitamin-A-deficient animals[7], the difference in the metabolic profile of AFB_1 by both categories of colon tissue may be a causative factor. This is probable in view of the fact that aryl hydrocarbon hydroxylase (AHH), cytochrome P-450 (or P-448) dependent enzyme metabolizes many polycyclic aromatic hydrocarbons (PAH), including AFB_1 to epoxides which lead to the formation of phenols, dihydrodiols, glutathione conjugates and covalently bound derivatives[16]. Although AHH-functions as a detoxication enzyme decreasing the carcinogenicity of PAH in the tissue, it may also be capable of activating PAH to carcinogenic, mutagenic or other toxic metabolites. It is now probable that the colon epithelium contains a pigment (other than cytochrome P-450) which has a higher activity in vitamin-deficient animals. The role of this form of pigment is being studied in the following regards:

a) Isolation and characterization;

b) probable role of the pigment as a terminal oxidase of PAH compounds, especially carcinogens turnover in the colon of the rat and baboons and

c) influence of dietary vitamin A on colon epithelial cytochrome levels in these species.

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Effect of sodium octanoate on leucine incorporation into protein of rat liver slices and of Yoshida ascites hepatoma cells

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Summary. 7.38×10^{-4} M octanoate does not significantly modify leucine incorporation into protein of rat liver slices, while in hepatoma cells a 19% inhibition has been noted. 3.69×10^{-3} M octanoate reduces leucine incorporation to about the same extent (71–76%) in both liver slices and hepatoma cells.

It has been reported that octanoate and other free fatty acids (FFA) exert an antitumor activity[1,2] that has been related to an inhibition of glycolysis[3] brought about by these substances in normal livers[3–5] or hepatomas[3]. It is also known that some inhibitors of protein synthesis, such as the aliphatic aldehydes, display an antitumor activity[6] with a poor effect on tissue respiration and glycolysis[7]. From the above data, it would appear interesting to observe if octanoate could modify protein synthesis of both normal and tumoral cells. This paper deals, therefore, with the effects of sodium octanoate on leucine incorporation into protein of liver slices of male and female normal rats and of ascites hepatoma cells.

Materials and methods. Liver slices (0.5 mm thick, 100–120 mg wet weight) from male and female normal rats or Yoshida ascites hepatoma cells (AH-130; 100–200 mg wet weight), obtained as previously described[8], were incubated with gentle agitation for 1 h, at 37 °C in a Dubnoff metabolic shaker, in 4 ml of Krebs-Ringer bicarbonate solution[9] containing 1 μCi of L-Leucine-^{14}C(U) (Radiochemical Centre Amersham U. K., spec. act. 331 mCi/mmole, 116 CPM/pmole) and the sodium octanoate concentrations reported in the table. The lower of these concentrations is in the range of the one found for total FFA in the plasma of our normal rats.

After incubation, the proteins of both liver slices and hepatoma cells were purified, and their radioactivity determined as previously described[8]. The statistical significance was evaluated with Student's t-test. When the comparison was made with material obtained from the same source, the significance was evaluated by the procedure for applying the t-test to the mean difference

Effects of sodium octanoate on L-leucine ^{14}C(U) incorporation into protein of liver slices of male and female rats and of Yoshida ascites hepatoma cells

Na-octanoate concentration in media	Liver of male rats	Liver of female rats	Hepatoma cells
0	8.926 ± 1.158 (5)*	9.850 ± 1.816 (5)*	37.718 ± 8.831 (5)*
7.38×10^{-4} M	9.745 ± 1.607 (4)	11.892 ± 3.026 (5)	30.506 ± 7.797 (5)[a]
0	8.926 ± 1.158 (5)**	7.328 ± 0.721 (5)**	30.130 ± 4.847 (4)**
3.69×10^{-3} M	2.608 ± 0.132 (5)[b]	1.916 ± 0.182 (5)[b]	7.262 ± 0.771 (4)[b]

Values are given as pmoles of leucine incorporated/min/mg protein and are the mean ± SE of the number of the determinations given in parentheses. Since the experiments with the 2 different octanoate concentrations have been undertaken at a different time for female rats and hepatoma cells, the figures for controls relative to 7.38×10^{-4} M and 3.69×10^{-3} M octanoate are marked with * and ** respectively. [a] $p < 0.02$; [b] $p < 0.01$ from appropriate control.