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 $\lambda_{max}$ 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.* $7.38 \times 10^{-4}$ M octanoate does not significantly modify leucine incorporation into protein of rat liver slices, while in hepatoma cells a 19% inhibition has been noted. $3.69 \times 10^{-3}$ 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)* |
| $7.38 \times 10^{-4}$ 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)** |
| $3.69 \times 10^{-3}$ 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 $7.38 \times 10^{-4}$ M and $3.69 \times 10^{-3}$ M octanoate are marked with * and ** respectively. [a] $p < 0.02$; [b] $p < 0.01$ from appropriate control.

of a series of paired varieties[10]. No statistical significance has been attached to differences with a probability value $p > 0.05$.

*Results.* The results are summarized in the table. Hepatoma cells exhibit higher levels of leucine incorporation into protein in comparison to liver slices ($p < 0.01$), while there are no significant differences between livers of male and female rats. $7.38 \times 10^{-4}$ M octanoate does not significantly modify leucine incorporation into protein of liver slices of rats of both sexes, while in hepatoma cells a 19% inhibition has been observed. $3.69 \times 10^{-3}$ M octanoate depresses protein synthesis about to the same extent in male (71%), female (74%) and hepatoma cells (76%).

*Discussion.* The higher levels of leucine incorporation into protein of hepatoma cells, in comparison with liver slices, is in line with previous results[8]. The greater susceptibility of protein synthesis of hepatoma cells to the lower concentration of octanoate could reflect a greater general sensitivity of tumors to exogenous FFA, since a defective feedback control of fatty acid synthesis has been demonstrated in hepatomas[11]. However, the different response could be also due to differences in the experimental models, i.e. isolated cells and slices.

It seems possible that the inhibition of protein synthesis by octanoate could be connected with that of glycolysis[3-5] and of mitochondrial respiration[12] previously found. The effect of octanoate on glycolysis has been obtained by preincubation with a cell-free supernatant fraction and, therefore, a direct extrapolation cannot necessarily be made with the results here reported. However it appears interesting to remember that a stimulatory effect by FFA on gluconeogenesis has been noted for tissue slices[13], perfused liver[14] and whole animal[15], and that this action has been connected to the inhibition of glycolysis[5]. The depression by octanoate of oxidation by rat liver mitochondria has been noted with much higher concentrations[12] than those used for the present experiments. However, it has been observed that a direct extrapolation cannot necessarily be made under the various experimental conditions. Namely the degree of micelle formation and protein binding is likely to differ for fatty acids on addition to cell-free systems, in the cell and in the whole organism[5].


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## Effect of the scorpion, *Heterometrus fulvipes* (C. Koch), venom on some enzyme systems in rat (albino) tissues


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*Summary.* Sublethal doses of *Heterometrus fulvipes* venom decreased NADP-specific isocitrate dehydrogenase and malate dehydrogenase activity levels and increased NADP-specific glucose-6-phosphate dehydrogenase and alkaline phosphatase activity levels during a 48-h-period.


The scorpion venom produced respiratory and cardiovascular arhythmia by its effect on cholinergic and adrenergic systems[2] Venom also caused alterations in NAD-specific dehydrogenases and hydrolytic enzyme systems both in vivo and in vitro conditions[3,4]. *Leiurus quinquestriatus* venom was known to produce linear inhibition of catalase activity in human erythrocytes[5], while *Buthus minax* venom inhibited acetylcholinesterase and succinate dehydrogenase activities in mouse tissues[6]. Hyperglycemic response due to venom action were also reported[7]. The present study reports changes in certain enzyme activities of metabolic significance due to sublethal doses of a less virulent type of the South Indian scorpion venon (*Heterometrus fulvipes*) in brain, sartorius and heart muscles, liver and serum of albino rats.

*Materials and methods.* Scorpion venom was collected by electrical stimulation. Crude and freshly collected venom was used. Protein content[8] was taken as criterion to express venom quantity. Male albino rats weighing 250 g were allowed free access to food and water, and $LD_{50}$ was determined by the method of Reed and Muenchi[9]. $^1/_3 LD_{50}$ was taken as sublethal dose. The animals were sacrificed after 6, 12, 24, 36 and 48-h-periods, and the tissue viz., brain, sartorius muscle, heart muscle and liver were quickly isolated at ice-cold temperature and homo-


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