(Radiochemical Centre, Amersham). The radioactivity is counted in a Packard scintillation counter 3320 for 10 min.

Results are recorded in the Figure. Liver cells in vitro produce de novo and secrete proteins with increasing intensity in the examined period as demonstrated by the values of labelled amino-acids incorporation into proteins recovered from the nutrient media. A remarkable decline of radioactivity is detected in the proteins isolated from the estradiol-17-β treated cultures media. In addition, the ^{14}C serine incorporation is affected to a lesser extent (55.8% of control values after 42 h and 70.9% after 66 h) than the ^{3}H leucine one (49.6 % after 42 h and 63.9% after 66 h). This fact could be explained with the synthesis in treated cells of phosvitin which contains approximately 30% by weight of serine[4].

Our data indicate that a correlation between the liver yolk proteins synthesis stimulation and the other proteins decreased production is present also in vitro. Researches are now in progress on the mechanism of this effect[5].

Riassunto. Culture primarie di fegato embrionale di pollo indotte a sintetizzare fosvitina con stimolo ormonico producono in minore quantità le altre proteine.

P. CARINCI, M. A. BODO, P. LOCCI and R. EVANGELISTI

Institute of Histology and General Embryology, University of Perugia, Via del Giochetto, I-06100 Perugia (Italy), 29 October 1973.

[4] S. E. ALLERTON and G. E. PERLMANN, J. biol. Chem. *340*, 3891 (1965).

[5] These studies were supported by Italian CNR Grant No. 70, 01069,04.

Carboxylic Acids as Carbon Sources for Aflatoxin Production

A correlation between free fatty acid concentration and aflatoxin production has been reported during the growth of *Aspergillus* strains on groundnuts. Aflatoxin production started only after the free fatty acids rose to a certain level[1]. There is thus a possibility of fatty acids being utilized as carbon sources for aflatoxin production. In the present study, the ability of different fatty acids, as well as other carboxylic acids, to act as carbon sources for aflatoxin production has been investigated using a synthetic medium developed in this laboratory.

Materials and methods. Aspergillus parasiticus strain NRRL 3240 used in the present study was obtained from Northern Regional Research Laboratory, Peoria, Illinois, USA. A spore suspension in double distilled water was prepared from 5- to 6-day-old cultures on glucose-peptone-agar and equally distributed to five 500 ml Erlenmeyer flasks containing 100 ml of sterile medium per flask. The synthetic medium (SLS medium) had the following composition: Sucrose 85 g; asparagine 10 g; ammonium sulphate 3.5 g; KH_2PO_4 10 g; $MgSO_4 \times 7H_2O$ 2 g; $CaCl_2 \times 2H_2O$ 75 mg; $ZnSO_4 \times 7H_2O$ 10 mg; $MnCl_2 \times 4H_2O$ 5 mg; $FeSO_4 \times 7H_2O$ 2 mg; ammonium molybdate $\times 4H_2O$ 2 mg and $Na_2B_4O_7$ 2 mg made up to 1 l in double distilled water. The pH of the medium was adjusted to 4.5 with HCl. *A. parasiticus* was grown at 26 $\pm$ 1°C for 8 days on a rotary shaker in the presence of different carboxylic acids which replaced sucrose from SLS medium on equivalent carbon (C) basis. Sucrose present in the SLS medium represents 35.8 g carbon/l. Similar experiments were also carried out by reducing the carboxylic acid level to provide 8.95 g carbon/l. In another experiments, sucrose was kept at 8.95 g carbon/l and different carboxylic acids were added as supplements to provide 8.95 g additional carbon/l. Insoluble carboxylic acids were left as fine suspensions. The pH of the medium was adjusted to 4.5 before sterilization.

On the 8th day of the growth, the mycelium and medium were separated, and dry weights of the mycelium determined. Aflatoxins from both medium and mycelium were extracted with chloroform and separated by thin layer chromatography on silica gel G using 2% methanol in chloroform. They were eluted with methanol and estimated by spectrophotometry using extinction coefficients reported by NABNEY and NESBITT[2]. Since the amounts of aflatoxins B_2 and G_2 were very low, aflatoxins

[1] U. L. DIENER and N. D. DAVIS, J. Am. Oil Chem. Soc. *44*, 259 (1967).

[2] J. NABNEY and B. F. NESBITT, Food Cosmet. Toxicol. *5*, 11 (1967).

Table I. Effect of different carboxylic acids on aflatoxin production

Carbon source	Mycelial dry weight (g/100 ml medium)	Aflatoxins (mg/100 ml medium)				
		In medium		In mycelium		Total
		B	G	B	G	
Sucrose	2.8	3.5	1.4	15.0	3.6	23.5
Sebacic acid	0.1	0.1	0.1	0	0	0.2
Lauric acid[a]	2.3	6.7	9.2	3.8	4.8	24.5
Myristic acid[a]	2.0	2.4	7.0	0.5	0.9	10.8
Palmitic acid[a]	1.5	0.4	0.3	0.4	0	1.1
Stearic acid	2.8	2.1	3.1	0.7	1.9	7.8
Oleic acid	1.9	1.6	0.8	1.2	0.7	4.3
Behenic acid[a]	0.5	0.1	0.1	0.1	0	0.3

[a] A part of the carboxylic acid was left unutilized after 8 days growth. Carbon sources were provided at a level of 35.8 g carbon/l medium.

Table II. Effect of different carboxylic acids on aflatoxin production

Carbon source	Mycelial dry weight (g/100 ml medium)	Aflatoxins (mg/100 ml medium)				Total
		In medium		In mycelium		
		B	G	B	G	
Sucrose	0.6	2.2	0.8	1.0	0.1	4.1
Sebacic acid	0.5	3.5	1.0	2.7	0.4	7.6
Lauric acid [a]	1.0	2.6	2.9	3.2	1.8	10.5
Myristic acid [a]	0.3	0.1	0.1	0	0	0.2
Palmitic acid [a]	0.5	0.1	0.1	0.1	0	0.3
Stearic acid	0.5	0.1	0.1	0	0	0.2
Oleic acid	0.6	0.1	0.1	0	0	0.2
Behenic acid [a]	0.3	0.1	0	0.1	0	0.2

[a] A part of the carboxylic acid was left unutilized after 8 days growth. Carbon sources were provided at a level of 8.95 g carbon/l medium.

B_1 and B_2 were estimated together as aflatoxin B and aflatoxin G_1 and G_2 together as aflatoxin G. Data presented are the average of 3 separate experiments.

Results and discussion. The results obtained on substituting the sucrose of SLS medium by different carboxylic acids on equivalent carbon basis are presented in Table I. Although lauric acid supported less growth, aflatoxin yields were comparable with those obtained with sucrose. With myristic and oleic acids, there was less growth, as well as less toxin production. DAVIS and DIENER[3] reported that when *A. parasiticus* was grown in stationary conditions on a medium containing 10% oleic acid and 2% yeast extract, aflatoxins were not synthesized, though good growth was obtained. Although stearic acid gave as much growth as sucrose, total aflatoxin yields were much less. Sebacic and behenic acids supported poor growth and only trace amounts of aflatoxins were formed. Acetic, propionic, butyric, caproic, heptoic, caprylic, nonoic, capric, glutaric and linoleic acids did not support any growth of the fungus. In contrast to the results obtained with sucrose, the ratio of aflatoxins in medium to those in mycelium was higher with all the carboxylic acids indicating greater excretion of the toxins from mycelium to medium.

Since high levels of some fatty acids can inhibit fungal growth, the above-mentioned experiment was repeated using a carbon (C) content of 8.95 g/l. At this level, sebacic and lauric acids supported higher aflatoxin production than sucrose. The increase in toxin production observed with sebacic and lauric acids was 85 and 156% respectively. With lauric acid, a marked increase in mycelial weight was also observed, but sebacic acid did not alter the extent of growth (Table II). The results obtained with sebacic acid are in marked contrast with those observed in Table I. Apparently sebacic acid at high concentrations inhibits growth. The observation of rather high yields of aflatoxin in presence of lauric acid is interesting. In this context, it is noteworthy that ARSECULERATNE et al.[4] obtained nearly 5 times more aflatoxins on coconut, which is rich in lauric acid[5], than wheat, rice or groundnut. Myristic, palmitic, stearic, oleic and behenic acids gave only trace amounts of aflatoxins. Again there was no growth and toxin formation with acetic, propionic, butyric, caproic, heptoic, caprylic, nonoic, capric, glutaric and linoleic acids.

In the experiments using SLS medium containing 21.25 g sucrose (8.95 gC)/l and supplemented with different carboxylic acids, sebacic acid gave much higher

[3] N. D. DAVIS and U. L. DIENER, Appl. Microbiol. *16*, 158 (1968).
[4] S. N. ARSECULERATNE, L. M. DE SILVA, S. WIJESUNDRA and C. H. S. R. BANDUNATHA, Appl. Microbiol. *18*, 88 (1969).
[5] A. P. DALE and M. L. MEARA, J. Sci. F. Agric. *6*, 162 (1955).

Table III. Effect of supplementing sucrose with different carboxylic acids on aflatoxin production

Supplementary carbon source equivalent to 21.25 g sucrose (8.95 g C)	Mycelial dry weight (g/100 ml medium)	Aflatoxin (mg/100 ml medium)				Total
		In medium		In mycelium		
		B	G	B	G	
Nil	0.6	2.1	0.7	1.2	0.2	4.2
Sucrose	1.4	2.9	1.2	5.1	1.3	10.5
Glutaric acid	1.2	1.6	0.5	3.8	1.0	6.9
Sebacic acid	1.1	2.8	0.8	8.2	2.1	13.9
Lauric acid [a]	1.3	1.6	1.0	4.7	0.9	8.2
Myristic acid [a]	1.1	2.2	1.2	2.4	0.8	6.6
Palmitic acid [a]	0.7	2.2	1.1	5.3	2.2	10.8
Stearic acid	1.1	1.4	1.0	1.3	0.4	4.1
Oleic acid	0.9	1.3	0.7	3.3	0.9	6.2
Behenic acid [a]	0.5	1.5	1.1	3.9	1.0	7.5

[a] A part of the carboxylic acid was left unutilized after 8 days growth. Both sucrose and the carboxylic acid were added at levels corresponding to 8.95 g carbon/l medium.

yields of aflatoxin, although mycelial weight was slightly less (Table III). Palmitic acid supported as much aflatoxin yield as that of control, although mycelial dry weight was reduced to half. With glutaric, lauric, myristic, stearic, oleic and behenic acids, aflatoxin yields were low. In almost all the cases, the amount of aflatoxin in the medium was higher than that in the mycelium. Even in the presence of sucrose in SLS medium, there was no growth or toxin production with acetic, propionic, butyric, caproic, heptoic, caprylic, nonoic, capric and linoleic acids. It may be noted that in the case of sebacic, myristic, palmitic, oleic and behenic acids, aflatoxin production is higher than the sum of the aflatoxin yields obtained on media containing sucrose and the carboxylic acid alone at a carbon level of 8.95 g/l.

Most of the carboxylic acids containing 2 to 10 carbon atoms, as well as linoleic acid, appear to be quite inhibitory. The fungistatic effects of acetic and propionic acids are well known, though their mode of action is not clear. In general, the effects observed with the higher carboxylic acids on aflatoxin production are dependent on the nature and concentrations of these acids. The fatty acids formed by the breakdown of the lipids present in natural substrates thus appear to have a significant influence on aflatoxin production by *Aspergillus*[6].

Résumé. On étudie l'effet de divers acides carboxyliques sur la production de l'aflatoxine en culture agitée d'*Aspergillus parasiticus* en milieu synthétique (SLS). Lorsque le saccharose correspondant à 35.8 g C/l de milieu SLS a été complètement remplacé par des acides gras à équivalence de C, seul l'acide laurique manifeste une croissance satisfaisante avec production d'aflatoxine. Quand le taux en C du milieu a été réduit à 8.95 C/l, les acides sébacique et laurique ont produit plus d'aflatoxine que le saccharose. Ajoutés en supplément à un taux peu élevé de saccharose dans le milieu SLS, les acides sébacique et palmitique ont eu un rendement élevé en aflatoxine.

S. R. GUPTA, H. R. PRASANNA, L. VISWANATHAN[7] and T. A. VENKITASUBRAMANIAN

Department of Biochemistry, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-7 (India),
29 March 1974.

[6] Acknowledgments: This investigation was supported by a PL-480 grant No. FG-In-438.
[7] Present address: National Sugar Institute, Kanpur, India.

Multiple Electrophoretic Bands of Serum Albumin in *Rana tigrina*

Albumin is a major serum protein in all vertebrates with the fastest anodal mobility in electrophoresis. It is believed to have no quaternary structure and in electrophoretic patterns always appears as a single major band. Two such bands appear in a heterozygote. Although genetic variants are known, variation is rare compared to some other polymorphic serum proteins like transferrin[1]. Phylogenetically also serum albumin is conservative in its electrophoretic location[2]. There have been relatively very few problems with non-genetic variation of this protein, although it has the ability to bind a variety of ions, lipids, drugs and hormones. It was thus surprizing that, during our studies on serum proteins of *Rana tigrina*, we have come across an extensive variation in both the location and number of electrophoretic bands of serum albumin. The possible nature of this variation is discussed.

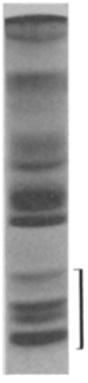

Fig. 1. Disc electrophoretic pattern of serum proteins of *R. tigrina*. The bands in bracket are albumins as identified by their mobility and binding to Evans Blue and hemoglobin.

Frogs (*Rana tigrina*) were caught from ponds and wells in and around the Warangal City, India. Blood was collected by puncturing the aorta close to the heart after mild etherisation of the animal. Serum was separated from the clotted blood the next day and electrophoresis carried out on the fresh sample. A simplified disc electrophoretic technique[3] with 5% acrylamide gel and *tris* buffer of pH 8.1 was used. 3 µl serum sample mixed in 1 ml of 20% sucrose was directly introduced on separation gel. 2.5 mA current per tube was allowed for the first 5 min and then the current raised to 5 mA and the run continued for 20 min. After electrophoresis, gels were stained with 1% amido-black for 30 min and the same were destained with 7% acetic acid overnight. For binding studies graded amounts of Evans Blue and Bromphenol Blue dyes were added to the serum samples and then electrophoresis was carried out. Only Evans Blue stained the albumin bands. Similarly freshly prepared hemoglobin solution was added to the sera and electrophoresis carried out. After electrophoresis, gels were stained with benzidine-peroxide solution[4]. Green bands corresponding to the albumin bands developed in 30 sec.

Figure 1 shows the disc-electrophoretic pattern of the serum proteins of *R. tigrina*. The fast moving components have been identified as albumin bands by their specific bonding with Evans Blue and hemoglobin and also by their electrophoretic behaviour. The 70 frogs examined so far have all shown this multiple banding. Depending on the number and relative positions, 10 patterns can be recognized (Figure 2). Identical patterns were obtained

[1] H. E. SCHULTZE and J. F. HEREMANS, *Molecular Biology of Human Proteins* (Elsevier, Amsterdam 1966).
[2] H. C. DESSAUER, W. FOX and Q. L. HARTWIG, Comp. Biochem. Physiol. *5*, 17 (1962).
[3] J. T. CLARKE, Ann. N.Y. Acad. Sci. *121*, 428 (1964).
[4] O. SMITHIES, Adv. Protein Chem. *14*, 65 (1969).