activity was variable with no difference between 20 P and 45 P rats. No CPK activity could be demonstrated in the liver.

The different dietary levels of protein resulted in variations in the liver activities of LDH, GPT and GOT similar to those described by SCHIMKE[6]. In animals on a high protein diet, an increase in the activity of the liver transaminases is part of a biochemical adaptation associated with increased amino acid metabolism. Likewise, the lower liver activity of LDH in these rats probably coincides with a decrease in the utilization of the glycolytic pathway.

Although the high protein diet induced much larger increases in liver GPT than in liver GOT, the differences in PGOT and PGPT were of opposite magnitude. This might be due to the varying cellular locations of the enzymes, or to their different plasma half lives.

There is a possibility that enzyme activities in other tissues were modified by the amount of dietary protein. However, it is likely that the higher levels of PGOT and PGPT in the 45 P rats were related in part, to the higher liver activities of these enzymes.

EGGLESTON and KREBS[11] have found 2 to 3-fold differences between the liver activities of 3 glycolytic enzymes in 4 strains of rats (3 Wistar derived; 1 Sprague Dawley) fed the same diet. Such apparent intrinsic strain differences in enzyme activity, together with variations induced by diet, could result in widely varying activities of many enzymes in both the liver and plasma. We feel, therefore, that considerable caution is warranted in the comparison of data on plasma enzyme activities obtained in different laboratories.

Zusammenfassung. Die Korrelation von Leberenzymen mit entsprechenden Plasmawerten wird diskutiert.

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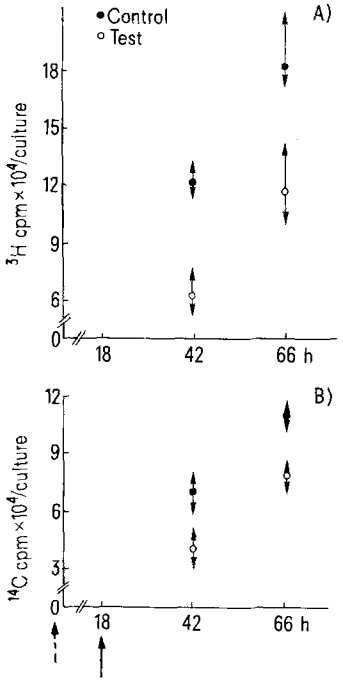
Decreased Synthesis of 'Secreted' Proteins in Chick Embryo Liver Cultures Treated by Estradiol-17-β

A decrease of all normal serum components accompanying a striking increase of liver-produced yolk proteins takes place in the laying hen or in both sexes following treatment with estrogen[1]. Since chick embryo liver primary cultures are able to produce and secrete into surrounding medium both serum proteins[2] and, after estradiol-17-β stimulation, a yolk protein, phosvitin[3], we have examined whether a similar effect can be observed in vitro. We have therefore studied the synthesis of total 'secreted' proteins by the estradiol-17-β treated liver cells after 42 h and 66 h in vitro i.e. during the period of phosvitin synthesis induction[3]. 14-day-old chick embryo liver cultures are performed as previously described[3]. Test cultures are supplied with nutrient containing 500 γ of estradiol-17-β (Merck; 50 γ/μl dissolved in propylene glycol) for 18 h. Media are then replaced and 2 independent sets of experiments are carried out. In the first experiment both control and hormone treated cultures are supplied with the nutrient containing 10 μCi/culture of L-(^{3}H)leucine (New England Nuclear Corporation; specific activity 38.58 Ci/mmole); in the second one with the nutrient containing 1 μCi/culture of L-(^{14}C) serine (New England Nuclear Corporation; specific activity 0.154 Ci/mmole). After collection and centrifugation (50 *g*/10 min) culture media are precipitated by 10% trichloroacetic acid, washed twice with 5% TCA, methanol-ethylic ether (1/1, v/v) and ether. Precipitates are then dissolved in about 1 ml of a protein solubilizer (soluene, Packard Instrument Co. Inc.) and mixed with 10 ml of spectrafluor PPO-POPOP scintillation fluid



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Effect of the administration of estradiol-17-β on the incorporation of ^{3}H leucine (A) and ^{14}C serine (B) into the total secreted proteins in the liver cultures. Each point represents the mean of determinations of 3 cultures; the vertical bars indicate the minimum and maximum values observed. 0 represents the experimental starting point (24 h after plating) dotted arrow the moment of estradiol-17-β administration and solid line the moment of hormone removal.

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(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.

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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

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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.