MEVALONATE PHOSPHORYLATION IN THE NEONATAL CHICK LIVER

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SUMMARY.- The phosphorylation of mevalonic acid by cell-free extracts from chick liver in the early steps of development was studied. A clear difference in the pH-activity profiles for phosphomevalonate and pyrophosphomevalonate formation has been observed. The amount of phosphomevalonate formed is quite similar at pH 7.5-9.5 whereas the pyrophosphomevalonate shows a clear maximum at pH 9.5. The pattern of mevalonate phosphorylation during the neonatal development shows no significant difference between 1-6 days after hatching, but a significant increase in the amount of phosphomevalonate formed at day 7 after hatching.

Cholesterol metabolism in the chick has received little attention though it is well known that it differs from that of mammals. Ovarian slices of the Gallus domesticus incorporate [1-^{14}C] acetate into progesterone, androstenedione, testosterone and estradiol-17β in the presence of gonadotrophin (1). The incorporation of [1-^{14}C] acetate into cholesterol by chick liver slices was enhanced by the feeding of raw soybean meal (2). Few studied have been conducted on mevalonic acid (MVA)* incorporation to cholesterol in avian livers. Nevertheless, the biosynthesis of cholesterol from MVA is well established in mammals, where the mevalonate kinase (EC 2.4.1.36) has been partially purified from hog liver (3,4), rabbit liver (5) and other sources.

The mechanism of regulation of cholesterol biosynthesis in rat liver has been elucidated. There is general agreement that

*Abbreviations: MVA, mevalonic acid; MVAP, phosphomevalonic acid; MVAPP, pyrophosphomevalonic acid; HMG-CoA, 3-hydroxy-β-methyl-glutaryl-CoA.

HMG-CoA reductase (EC 1.1.1.34) is the rate-limiting enzyme in cholesterol synthesis. However MVA kinase may play an important role in the regulation of this process. Dorsey and Porter (6) have shown that geranyl-PP and farnesyl-PP are powerful inhibitors of MVA kinase from pig liver, suggesting that both terpenyl pyrophosphates act as physiological controls after MVA.

In plants, the terpenoid biosynthesis seems to be controlled by a combination of enzyme segregation within the cell and specific impermeability of the intracellular membranes to certain compounds (7). In French-beans (8) and pumpkin seedlings (9) the presence of two isoenzymes of mevalonate kinase have been demonstrated, one located in the chloroplasts and the other one outside them, with an optimum pH of 7.5 and 5.5 respectively. We have isolated two fractions with MVA kinase activity from Pinus pinaster and Agave americana, both fractions being active at pH 7.9 (10).

As an intent to elucidate the role of MVA kinase in the regulation of cholesterol biosynthesis, we have studied some aspects of MVA phosphorylation in the chick liver in the early steps of development.

MATERIALS AND METHODS

New-born White Leghorn male chicks were obtained from a commercial hatchery and maintained on a commercial diet. [2-^{14}C] MVA was supplied as the lactone by the Radiochemical Centre, Amershan, England. The potassium salt was prepared by treating the lactone at 36º for 30 min with an excess of KOH.

Cell-free extracts were obtained by homogenizing the liver in a Potter-Elvehjem homogenizer with 0.05 M Tris-maleate buffer, pH 7.5, so that the final liver/buffer ratio is 1/10. The homogenate was centrifuged at 15000 g for 30 min at 4º. Protein content in the supernatant was determined by the Lowry method (11).

Unless otherwise specified, the reaction system contained 6 µmoles of $MgCl_2$, 12 µmoles of ATP, 75 nmoles of [2-^{14}C] MVA, 150 µmoles of Tris-maleate buffer and 0.2-0.5 mg of protein in a final volume of 1.5 ml. The reaction mixture was incubated at 37º for 30 min. Reactions were stopped by heating the tubes to 90º for 5 min. Aliquots (25 µl) of supernatants were applied to Whatman No 1 paper strips and developed by the ascending technique in butanol-formic acid-water (77:13:10). Radioactive spots on the dried strips were detected and measured in a Nuclear-Chicago Actigraph III system.

RESULTS AND DISCUSSION

MVA phosphorylation by a cell-free extract from chick liver has been investigated in reaction carried out for 0-180 min, showing that MVAP formation occurs linearly between 0-30 min, whereas MVAPP formation is linear as far as the reactions are prolonged to 90 min (Fig. 1). The maximum amount of MVAPP found is about 50 % that MVAP formed.

On the other hand, we have studied the MVA phosphorylation by adding 0-2.5 mg protein in the reaction mixture, showing that in the conditions described in Methods the MVAP formation is proportional to the amount of protein added over the range 0-0.5 mg, whereas the amount of MVAPP formed increases linearly to 1.5 mg protein (Fig. 1). It is important to note that in any case the MVA phosphorylation is not dependent on the presence of protector of -SH groups. Addition of 10^{-3} M cysteine has no effect.

The incorporation of MVA by cell-free extracts from chick liver has been studied over the range pH 6.5-9.5 in 0.05 M Tris-

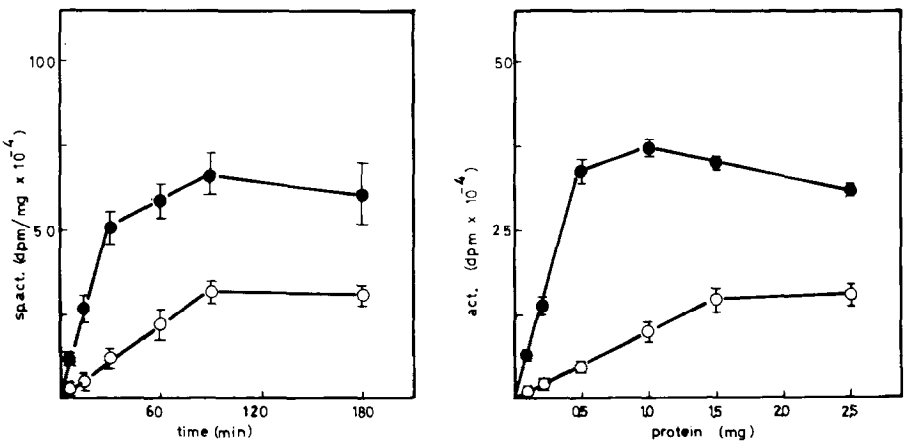

Fig. 1.- Influence of incubation time and protein concentration on MVA phosphorylation by cell-free extracts from chick liver. The results are given as means of six experiments ± S.E.M. Experimental details are given in the text. ●, MVAP; ○, MVAPP.

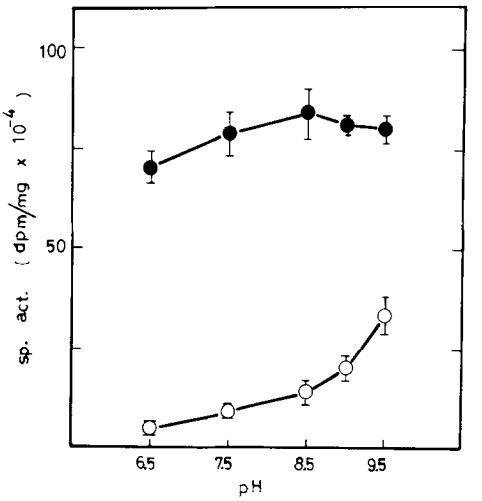

Fig. 2.- Effect of the pH on the MVA phosphorylation by cell-free extracts from chick liver. The results are given as means ± S.E.M. of six experiments. Experimental details are given in the text. ●, MVAP; ○, MVAPP.

maleate buffer. A clear difference has been observed in the pH-activity profiles for MVAP and MVAPP formation (Fig. 2). The MVAP formation is quite similar at pH 7.5-9.5 whereas the MVAPP shows a clear maximum at pH 9.5. The amount of MVAPP at this pH value is almost 4-fold than that obtained at pH 7.5. These results differ from those reported by Hellig and Popjak (12) for the pig liver MVAP kinase, which has an optimum pH of 7.3, unlike the similar enzyme from yeast which is equally active in the range pH 5.3-10.0 (13). The difference observed between the optimum pH of MVAP kinase and the pH of its intracellular surroundings seems to suggest a system to control this enzyme activity.

The pattern of MVA phosphorylation during the neonatal development is shown in Fig. 3. No significant differences have been found between 1-6 days after hatching. A significant increase in the amount of MVAP formed has been found at day 7 after hatching. Working also with Gallus domesticus Gomez-Capilla et al. (14) have shown a clear decrease in the total cholesterol at day 5 and 10 after

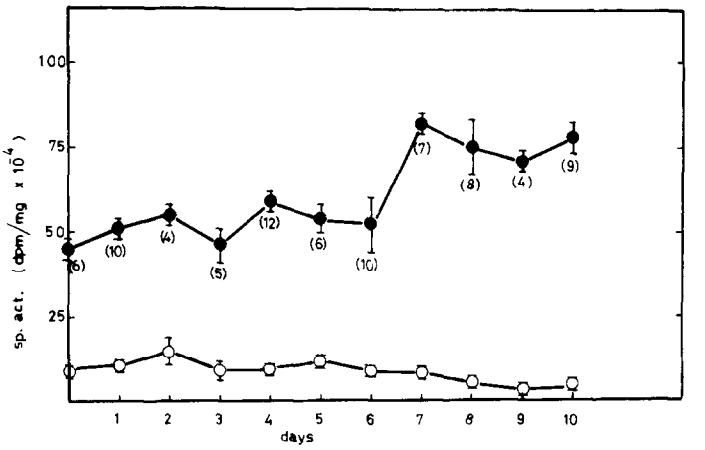

Fig. 3.- MVA phosphorylation by cell-free extracts from chick liver during the neonatal development. The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text. ● , MVAP; ○ , MVAPP.

eclosion, a decrease which does not seem to be parallel to the MVA phosphorylation ability in this period. On the other hand, little is known about the development pattern of mevalonate kinase in animal sources. Only Barnes and Goodfellow (15) have determined some developmental fluctuation in the MVA kinase activity from larva, pharate adult and adult of Sarcophaga bullata.

Recently, Ramachandran and Shah (16) have studied the phosphorylation and decarboxylation of mevalonate by 105000 g supernatant fraction from livers of suckling and weaned rats, showing that MVAPP decarboxylation is one rate-limiting step in the hepatic cholesterol synthesis.

Although cell-free extracts have been used in all the experiments, the MVA activating enzymes are essentially located in the soluble fraction (Table 1). The microsomal location of the chick liver HMG-CoA reductase (unpublished results) suggests another regulation system of the cholesterol metabolism in this source.

Table 1

Intracellular distribution of MVA-activating enzymes from chick liver.

	Specific activity (dpm / mg protein)	
	MVAP	MVAPP
Crude homogenate	31.99 ± 1.21	3.32 ± 0.30
15000 g pellet	6.24 ± 0.48	-
105000 g supernatant	75.22 ± 2.03	9.21 ± 0.66
105000 g pellet	13.79 ± 0.61	-

Results are given as means ± S.E.M. of three experiments.

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