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Squalene, Lanosterol and Cholesterol Synthesis from Acetate in Neonatal Chick Tissues

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Acetate incorporation into squalene, lanosterol and cholesterol by liver and kidney slices and intestinal mucosa scrapes from neonatal chick was studied. Contrary to what is observed when using mevalonate as substrate, cholesterol was the main nonsaponifiable synthesized from acetate in all the conditions assayed. Low percentages of squalene and lanosterol were synthesized by liver and kidney slices, while in intestinal mucosa squalene was practically undetectable. The highest percentage of radioactivity in cholesterol was found in liver, followed by intestinal mucosa and kidney. Relative percentages of squalene, lanosterol and cholesterol were practically similar in each tissue at any incubation time and acetate concentration considered. Only in kidney these percentages, especially in the case of squalene, seemed to decrease at higher acetate concentrations (8-12 mM).

Key words: Sterol biosynthesis, Acetate, Neonatal chick.

Although every tissue possesses the ability to synthesize cholesterol, the relative rate of cholesterogenesis in various tissues of a number of species has not yet been properly established. There is a general agreement that liver and small intestine are the major anatomical sites of cholesterol synthesis (12). However, a number of observations suggest that relative rates of cholesterogenesis are related to the substrate used for measuring this process. Thus, in a previous paper we have reported that acetate incorporation into nonsaponifiable lipids was maximal in neonatal chick liver, whereas kidney and intestinal mucosa only showed about 10 % of the hepatic cholesterogenic activity (4). Nevertheless, the incorporation of mevalonic acid (MVA) into nonsaponifiable lipids by neonatal chick was higher in kidney than in liver (1), corroborating the main role of kidney in MVA metabolism (7, 8, 14).

Hepatic cholesterol synthesis appears to be primarily regulated at the site of the reaction catalyzed by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase by different mechanisms (5, 9, 11). There is a high correlation between HMG-CoA reductase activity and the overall rate of acetate incorporated into cholesterol in various physiological states (3, 6, 11).

The object of the present study was to examine possible differences between the ability of neonatal chick tissues to synthesize cholesterol and its precursors, squalene and lanosterol, from acetate.

Materials and Methods

Newborn White Leghorn male chicks (*Gallus domesticus*) were obtained from a commercial hatchery and maintained fed *ad libitum* on a commercial diet (Sanders A-00) in a chamber with a light cycle from 09.00 to 21.00 hr and controlled temperature. Ten-day-old chicks were used. Chicks were killed by decapitation at the same hour every day (8 a.m.). [1-^{14}C]-acetate and [7-^{3}H]-cholesterol were supplied by the Radiochemical Centre, Amersham, U. K. All other reagents used were analytical grade.

Procedures for determining the conversion of [1-^{14}C]-acetate into nonsaponifiable lipids were based on those described by RIGHETTI *et al.* (10) in studies on the *in vitro* MVA metabolism, with the modifications described by ARCE *et al.* (4). The nonsaponifiable material was fractionated by thin-layer chromatography and identified as previously described in detail (2). All experiments were carried out in duplicate and at least two determinations were done in each experiment. Results are expressed as relative percentages of squalene, lanosterol and cholesterol in the nonsaponifiable fraction. The variation in the obtained values did not exceed 10 %.

Results and Discussion

The time course of the formation of the different nonsaponifiable lipids by neonatal chick liver, kidney and intestinal mucosa was established by a series of experiments carried out *in vitro* at different incubation times (15-120 min) in the presence of 3 mM acetate. The relative percentages of squalene, lanosterol and cholesterol were practically similar in each tissue at any incubation time considered (table I), notwithstanding the fact that the rate of acetate incorporation in the total nonsaponifiable fraction by the three tissues was essentially linear with respect to the incubation time assayed (4). In no tissue did the radioactivity in squalene, lanosterol and cholesterol totalize 100 % of the radioactivity in the nonsaponifiable fraction. However, the identity and percentages of these compounds were well established by using internal standards in the thin-layer chromatography technique. A non-identified fraction resting at the origin of the TLC plates was also separated by this procedure. The nature of this type of polar nonsaponifiable lipid(s) is currently under study. The expressed percentage of squalene includes the oxygenated squalene and possibly the synthesized ubiquinone. Likewise, cholesterol percentage includes C-27 sterols other than cholesterol.

It is important to remark the low percentages of squalene and lanosterol synthesized by liver and kidney slices; even more, in intestinal mucosa squalene was practically undetectable. These results contrast with those obtained by using MVA as substrate in neonatal chick, in which squalene was by far the major nonsaponifiable syn-

Table I. *Percentage of radioactivity in squalene, lanosterol and cholesterol in the nonsaponifiable fraction obtained at different incubation times in the presence of 3 mM [1-^{14}C] acetate.*

All experiments were carried out in duplicate with pools of 4 animals and at least two determinations were done in each experiment. The obtained values agreed within 10 % of each other. Nd, non detected.

Incubation time (min)	Liver			Kidney			Intestinal mucosa		
	Squalene	Lanosterol	Cholesterol	Squalene	Lanosterol	Cholesterol	Squalene	Lanosterol	Cholesterol
15	3.82	4.32	45.33	0.84	1.50	23.97	Nd	Nd	40.06
30	2.66	4.48	55.28	1.50	4.73	27.28	Nd	4.70	37.56
60	1.13	1.38	46.58	6.81	3.52	29.76	Nd	5.93	32.68
90	4.14	2.51	56.16	3.94	3.02	26.03	Nd	7.91	35.32
120	3.49	3.05	59.46	5.13	3.61	16.60	1.77	5.98	44.03

Table II. *Influence of [1-^{14}C] acetate concentration on the percentage of radioactivity in squalene, lanosterol and cholesterol in the nonsaponifiable fraction obtained after 2 hr of incubation.*

All experiments were carried out in duplicate with pools of 4 animals and at least two determinations were done in each experiment. The obtained values agreed within 10 % of each other. Nd, non detected.

Acetate [mM]	Liver			Kidney			Intestinal mucosa		
	Squalene	Lanosterol	Cholesterol	Squalene	Lanosterol	Cholesterol	Squalene	Lanosterol	Cholesterol
1	2.41	2.75	59.47	12.43	15.60	45.58	1.05	8.47	41.23
2	1.86	2.32	52.06	17.97	15.68	33.03	1.12	9.29	39.51
3	1.97	2.14	58.52	8.01	9.38	32.45	Nd	6.94	27.94
8	2.86	2.21	60.26	Nd	11.32	9.43	Nd	3.76	55.05
12	2.82	1.05	58.20	Nd	10.94	22.67	Nd	7.03	47.28

thesized by kidney (2). Likewise, a significant portion of nonsaponifiable radioactivity was found in squalene and methyl sterols in isolated small-intestinal cells of the rat (13). Thus, after 2 hr of incubation 29 % and 44 % of radioactivity from [1-^{14}C]-acetate was found respectively in squalene and methyl sterols of villous cells and 19 % and 33 % in the crypt cells, values clearly higher than those found in chick intestinal mucosa.

In a previous work we have reported that acetate incorporation into the total nonsaponifiable fraction by liver slices plateaued at essentially constant values at substrate concentrations of approximately 6 mM whereas the incorporation by kidney and intestinal mucosa increased in a nearly linear relationship to the acetate concentration until 12 mM (4). In order to determine the possible influence of substrate concentration on distribution of radioactivity among the different nonsaponifiable lipids, a series of experiments were carried out in which increasing amounts of [1-^{14}C]-acetate ranging from 1 to 12 mM were incubated with liver and kidney slices and intestinal mucosa scrapes. The percentages of squalene, lanosterol and cholesterol were practically independent from acetate concentration in liver and intestinal mucosa (tabla II), while in kidney these percentages, especially in the case of squalene, seemed to decrease at higher acetate concentrations (8-12 mM). Small differences in the percentages of different nonsaponifiable lipids were observed in various experiments carried out in the same conditions, mainly due to variations in the amount of the non-identified polar nonsaponifiable fraction. In any case, these small differences are not significant enough to change the meaning of the obtained results.

These results contrast again with those obtained by using MVA as substrate in which the percentage of squalene synthesized in kidney clearly increased with MVA concentration reaching more than 50 % from 0.8 mM MVA onwards (2). The high proportion of squalene recovered from MVA in kidneys could be explained by the slow conversion of this intermediate to cholesterol in the renal tissue. However, 6 hours after the injection, the major end product of MVA metabolism in liver and kidney was cholesterol (2) demonstrating that, with time, neonatal chick kidneys are also readily capable of carrying the process of cholesterol synthesis to completion. Other regulatory mechanisms exerted by different sterol precursors or their derivatives on the squalene metabolizing enzymes could be suggested. In any case, the physiological significance of the role of liver, kidney and intestinal mucosa in the conversion of acetate and MVA to squalene, lanosterol and cholesterol is under study, in view of the influence of the substrate nature and the method used for measuring the rate of synthesis of cholesterol and its precursors.

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Resumen

Se estudia la incorporación de acetato a escualeno, lanosterol y colesterol en cortes de hígado y riñón y en raspados de mucosa intestinal de pollo recién nacido. Al contrario de lo observado usando mevalonato como substrato, el colesterol es el principal insaponificable sintetizado a partir de acetato en todas las condiciones ensayadas. En cortes de hígado y riñón

se sintetizan bajos porcentajes de escualeno; en mucosa intestinal el escualeno es prácticamente indetectable. El porcentaje más alto de radioactividad recuperada como colesterol se encuentra en el hígado, seguido por la mucosa intestinal y el riñón. Los porcentajes relativos de escualeno, lanosterol y colesterol son prácticamente semejantes en cada tejido, cualquiera que sea el tiempo de incubación y la concentración de acetato considerados. Sólo en riñón parecen disminuir estos porcentajes a las concentraciones más altas de acetato (8-12 mM), especialmente en el caso del escualeno.

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