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INHIBITION OF CHOLESTEROL BIOSYNTHESIS BY THE RESPIRATORY CHAIN INHIBITORS IN HUMAN PLACENTA AND IN RAT LIVER

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Abstract—The inhibition of [^{14}C]acetate incorporation in the presence of amytal, rotenone and cyanide in squalene and cholesterol fractions was observed in placenta and liver tissue.

THE BIOSYNTHESIS of cholesterol in liver cell is localised in the microsomal and cytoplasmic fractions.[1] NADPH and ATP pool is one of the most important factors regulating this process.[2] The level of these nucleotides depends mainly on the function of mitochondria. Besides, the concentration of acetylo-CoA in the cell is also dependent on the metabolism of mitochondria. Therefore one could expect that the function of respiratory chain, in not enriched environment, has some effect on the cholesterol biosynthesis. Żelewski and Villee[3] showed that large differences exist between acetate and mevalonate incorporation ratio into squalene, lanosterol and cholesterol in placenta. They found that the incorporation of [^{14}C]acetate and [^{3}H]mevalonate expressed as the ^{3}H:^{14}C ratio was much greater for lanosterol, and for cholesterol was much smaller than the ratio for squalene. It would be interesting to know whether the biosynthesis of sterols in placenta and liver is related in some way to the mitochondrial respiratory chain, and if so, whether this relation is similar for the squalene, lanosterol and cholesterol biosynthesis. In the present work the effect of respiratory chain inhibitors: amytal, rotenone and KCN, on the incorporation of [^{14}C]acetate into squalene, lanosterol and cholesterol has been studied.

METHODS

Human term placentas obtained immediately after delivery and livers obtained from mature Wistar strain rats were cut into small slices, washed three times with 0·9% NaCl and once with Krebs–Ringer phosphate buffer pH 7·4. Ten g of placenta tissue or 5 g of liver were incubated in 10 or 5 ml of Krebs–Ringer phosphate buffer containing 125 μc or 62·5 μc [^{14}C]2-acetate (38 mc/m-mole) respectively. The experiment was carried out in four separate flasks containing: (1) no inhibitors-control, (2) amytal 3 mM, (3) rotenone 1 μM and (4) KCN 2 mM. The tissues were incubated in Erlenmeyer flasks shaking at 37° in oxygen atmosphere for 2 hr. The incubations were terminated by cooling the flasks. After the incubation, minced tissue was separated from incubation medium, transferred to a homogenizer and 10 mg of cholesterol, 10 mg of lanosterol and 20 mg of squalene was added as carrier. The tissue was homogenized twice in 20 ml of an ethanol–acetone–ether 4:4:1 mixture and once in

20 ml acetone, ethyl acetate and ether. After every extraction the homogenate was filtered through filter paper with rinsing. The filtrates were combined, evaporated and the residue was saponified in 15% potassium hydroxide and methanol at 60° for 1·5 hr. The solution was then neutralised with glacial acetic acid, using phenolphthalein as an external indicator. The saponified extract was partitioned between hexane and 90% methanol by a five-tube counter current distribution. The hexane fraction was evaporated, residue was chromatographed on thin layer plates of silica gel impregnated rhodamine 6G in the system benzene–ethyl acetate (5:1). Three fractions were obtained. The squalene fraction was further purified on TLC in the systems: of chloroform, benzene–ethyl acetate (19:1) and cyclohexane. The lanosterol and cholesterol fractions were rechromatographed in the following systems: ethyl acetate–hexane (25:75), benzene–methanol (93:7), chloroform and cyclohexane–ethyl acetate (49:51). The cholesterol was then further purified by bromination followed by regeneration with zinc. The cholesterol and lanosterol were finally crystallised three times from methanol–acetone, methanol–methylene chloride and methanol. Samples were dissolved in 5 ml of scintillation fluid containing 4 g of 2,5 diphenylooxazole and 0·2 g of 1·4 di[2(5-phenyloxazolyl)]benzene per liter of toluene, and were assayed in liquid scintillation counter. Total sterols were assayed with the Liebermann–Burchard color reagent. Squalene was assayed by the methods of Rothblat.[4] The values are expressed as counts/min/mg. The endogenous content of cholesterol in the human placenta and in rat liver was 1·8 mg and 2 mg/g tissue respectively. The amount of carrier squalene and lanosterol exceeded many times the endogenous level of these substances.

RESULTS

The results from two typical experiments presented in Table 1 show that amytal, rotenone and cyanide caused a marked decrease of incorporation of [^{14}C]acetate by minced human term placenta into the squalene and cholesterol fractions.

The fall of specific radioactivity in the presence of inhibitors was greater in cholesterol fraction than in squalene. Comparison of the effect of inhibitors on the incorpora-

TABLE 1. THE INCORPORATION OF [^{14}C]ACETATE INTO SQUALENE, LANOSTEROL AND CHOLESTEROL BY PLACENTAL TISSUE IN THE PRESENCE OF AMYTAL, ROTENONE AND CYANIDE

Flask	Inhibitors	Squalene (counts/min/mg)	(%)	Lanosterol (counts/min/mg)	(%)	Cholesterol (counts/min/mg)	(%)
Expt. I*							
1.	none	300	100	1100	100	1700	100
2.	amytal	100	33	1200	109	180	11
3.	rotenone	90	30	900	82	450	26
4.	cyanide	80	27	1800	164	100	6
Expt. II							
1.	none	280	100	3060	100	790	100
2.	amytal	70	25	1460	48	15	2
3.	rotenone	100	36	1580	52	50	6
4.	cyanide	80	28	2730	89	12	1·5

Results are expressed as counts/min/mg and per cent of the control.
* In Expt. I fraction of cholesterol was not brominated.

tion of [^{14}C]acetate into lanosterol fraction with that into squalene, indicates that the effect of any inhibitor used is much more pronounced for squalene which is a precursor of lanosterol. Table 2 presents the data from the experiments carried out with rat liver tissue. The inhibitory effect of amytal, rotenone and cyanide on the incorporation of [^{14}C]acetate into squalene and cholesterol was very similar to those obtained with placenta tissue. Here again the fall in specific radioactivity in the presence of inhibitors is more marked for squalene than lanosterol fraction.

TABLE 2. THE INCORPORATION OF [^{14}C]ACETATE INTO SQUALENE, LANOSTEROL AND CHOLESTEROL BY LIVER TISSUE IN THE PRESENCE OF AMYTAL, ROTENONE AND CYANIDE

Flask	Inhibitors	Squalene (counts/min/mg)	(%)	Lanosterol (counts/min/mg)	(%)	Cholesterol (counts/min/mg)	(%)
Expt. I							
1.	none	2400	100	510	100	37,000	100
2.	amytal	1500	62	—	—	10,000	27
3.	rotenone	1300	54	440	86	10,000	27
4.	cyanide	210	9	170	33	1870	5
Expt. II							
1.	none	1700	100	1500	100	46,000	100
2.	amytal	720	42	1200	80	12,000	26
3.	rotenone	900	53	800	53	22,600	49
4.	cyanide	90	5	300	20	890	2

Results are expressed as counts/min/mg and per cent of the control.

DISCUSSION

The experimental results presented here provide evidence for an inhibitory effect of amytal, rotenone and KCN on the biosynthesis of squalene and cholesterol from acetate by placental and liver tissue. From the specific radioactivity of squalene, lanosterol and cholesterol one may conclude that the inhibitors used decreased the conversion of acetate to squalene and of lanosterol to cholesterol. As in both tissues investigated the specific radioactivity of lanosterol changed in a smaller extent under the influence of inhibitors than the specific radioactivity of squalene or cholesterol, no conclusions can be drawn about the influence of inhibitors on the conversion of squalene to lanosterol.

Gaylor[5, 6] showed that arsenite decreases incorporation of [^{14}C]mevalonate into squalene and cholesterol, but not into lanosterol fraction in rat skin. Incorporation of [^{14}C]acetate into all three fractions was decreased under the same conditions. He explained these results by assuming that arsenite inhibits the reaction sequence between acetate and mevalonate, between lanosterol and companions and cholesterol, to a lesser extent between mevalonic acid and squalene and only slightly between squalene and lanosterol and companions. The effects obtained while using amytal, rotenone and cyanide are similar to those described by Gaylor. The chemical structure of these compounds is quite different and it seems possible that the identical inhibitory effect on the biosynthesis of squalene, lanosterol and cholesterol is due to the fact that all of them are inhibitors of respiratory chain.

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