INCORPORATION OF 1-14C ACETATE INTO LIPIDS BY SIX SUCCESSIVE GENERATIONS OF EHRLICH ASCITES TUMOR CELLS AND THE LIVERS OF THE TUMOR-BEARING MICE

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Cancer researchers use Ehrlich ascites tumor cells as a very convenient tool for basic studies and chemotherapy screening and clinicians regard them as prognostically significant. These tumor cells which are relatively free-growing **in vivo** can be readily obtained without excessive handling and therefore these represent convenient model tumor systems for study.

The peculiar properties of tumor cells may be dependent to a large extent on the properties of their membrane (Coman, 1953; Abercrombie and Ambrose, 1962) which in turn may be dependent mainly on its chemical composition. The major biochemical components of the membrane are proteins and phospholipids. The importance of lipids, especially phospholipids, in cell membrane and in the structure of such subcellular particles as mitochondria is well recognised. This fact has stimulated some studies on the phospholipid metabolism and composition of tumor tissues (Begg and Trew, 1957; Gray, 1963). Phosphatides have long been suspected of having special relation to the genesis of tumor (Jablonski and Olson, 1955). A quantitative study of the phospholipids of Ehrlich ascites tumor cells was made by Wallach, Soderberg and Bricker (1960). Lee *et al* (1962) have concluded that the rates of lipogenesis in tumors are different from that of normal cells whereas Meads and Weinhouse (1958) have reported that the metabolism of lipids in tumors is not different from that of other tissues.

The present study of the lipid synthesis in six consecutive generations of ascites tumor cells, the livers of the tumor-bearing mice (host-livers) and that of normal mice of the same species was undertaken with a view to compare the incorporation of acetate 1—14C into the various lipid fractions by the above three types of cells. The study was limited to the quantitative determination of 1—14C acetate uptake for the purpose of incorporating it into cholesterol, phospholipids and fatty acids.

MATERIALS AND METHODS

Ascites tumor cells cultured asceptically in white Swiss mice were used for the work.

The tumor cells were removed using sterile syringes from the peritoneal cavity of the mice on the eleventh day after transplantation. The fluid containing the cells were spun out for 3 minutes at 2500 rpm., the supernatant discarded and the cells resuspended in 5 ml. of Krebs ringer bicarbonate buffer (pH 7·4) and recentrifuged. The procedure of washing the cells with bicarbonate buffer was repeated twice. One part of the cell sediment was suspended in four parts of the buffer and kept at 0°C. for use. One ml. of this suspension had 24·6 ± 1·1 mg. cells by dry weight.

Livers of the tumor-bearing mouse (host liver) and normal mouse of the same species, having the same age were also sliced and kept in ice-cold buffer at 0°C. until used.

500 mg. of the liver slices from normal and host mice and 2 ml. of tumor cell suspensions were incubated separately in duplicate with 4 wc of acetate 1-14C, so as to give a total volume of 5 ml. after the addition of Krebs ringer bicarbonate buffer. The incubation was carried out in a Dubnoff shaker bath at 37·5± 0.5°C. for 3 hours, using air as gas phase. At the end of the incubation the contents were transferred into separate centrifuge tubes, spun at 2500 rpm for 5 minutes and the supernatant discarded. The sediment was resuspended in 5 ml. of distilled water, centrifuged and the supernatant discarded. The process was repeated two more times.

Consecutive generations of tumor cells were produced from the preceding ones by culturing the tumor cells in white Swiss mice and all generations of tumor cells and the liver slices of the tumor-bearing mice and that of the normal mice were incubated separately as described above.

Total lipid extractions were carried out according to a procedure similar to those of Folch, Lees and Sloan-Stanley (1957) and

Wren & Mitchell (1959). The total lipids were separated into acetone precipitable, digitonin precipitable and free fatty acids of neutral fat fractions according to a modified procedure (Bhattathiry, 1966). The acetone precipitable fraction (phospholipids) was further separated into cold alcohol soluble, diethyl ether soluble and hot alcohol soluble fractions. These fractions roughly correspond to lecithin, cephalin and sphingomyelin respectively. The remainder which was insoluble in alcohol or ether was preserved. The free fatty acids from phospholipids were isolated and then methylated according to the methods of De Boer (1956) and Schlenk & Getterman (1960). These methyl esters of fatty acids were separated into saturated, mono, di and tri ethylenic, using a method similar to that of De Vries (1963).

Radioactivity of all samples was determined in a windowless gas flow counter, as infinitely thin film preparations (Bhattathiry, 1966) and activities thus obtained were corrected for self-absorption.

RESULTS

Table I shows the incorporation of 1-14C acetate into the phospholipids, fatty acids, and cholesterol by 500 mg. each of normal and tumor-bearing mice liver slices and 2 ml. of the ascites tumor cell suspensions. Radioactivity is expressed as counts per minute. In this table the numbers 1 to 6 in the first column refers to six generations as far as the tumor cell suspensions are concerned.

The total phospholipids were separated into cold alcohol soluble (lecithin,) ethyl-ether soluble (cephalin), hot alcohol soluble (sphingomyelin) and a fourth fraction which was insoluble in all of these solvents. Table II represents the result of analysis of the radioactivity in the different phospholipid fractions. The results are expressed as percentage of the total radioactivity in each fraction of the phospholipids.

Total phospholipids from all the six series of experiments of livers from normal and tumor-bearing mice as well as ascites tumor cells were

TABLE I

RADIOACTIVITY (TOTAL COUNTS PER MINUTE) OF PHOSPHOLIPIDS, CHOLESTEROL AND FATTY ACIDS FROM LIVERS OF NORMAL AND TUMOR —BEARING MICE AND ASCITES TUMOR CELL SUSPENSIONS

Exp.		Liver from normal mouse	Liver from tumor-bearing mouse	Tumor cell suspension
1	PL	31650	49650	17850
	Chl	18950	31650	2150
	FA	47700	84200	7100
2	PL	35550	65750	34300
	Chl	12400	38900	2650
	FA	45200	78900	9750
3	PL	31000	58000	31300
	Chl	16750	29250	2050
	FA	59550	89100	7600
4	PL	34100	52350	21100
	Chl	18600	28800	2150
	FA	60300	106050	6500
5	PL	29750	45950	19450
	Chl	21900	32500	2500
	FA	52800	92300	7000
6	PL	32350	45700	16250
	Chl	15950	24750	2700
	FA	53150	97900	6200

PL = Phospholipids; Chl = Cholesterol; FA = Fatty acids.

TABLE II

PERCENTAGE OF RADIOACTIVITY IN INDIVIDUAL PHOSPHOLIPIDS

	Cold alcohol soluble fraction %	Ethyl-ether soluble fraction %	Hot alcohol soluble fractions %	Remainder %
Livers from normal mice	32.2	65.3	1.0	1.5
Livers from tumor-bearing mice	37.8	60.7	0.6	0.9
Tumor cells	12.8	84.8	1.7	1.7

pooled separately and the fatty acids isolated and methylated. The methyl esters of free fatty acids from each group were separated into saturated, mono, di and tri ethylenic fatty acids and their radioactivity determined. Table III shows the result of this analysis.

DISCUSSION

The livers of the tumor-bearing mice in all the six cases, have shown a greater ability to incorporate 1-14C acetate into the total phospholipids, cholesterol and fatty acids of the neutral fats when compared with livers from normal mice. This ability ranged from 1·5 to 2 times in the case of fatty acids (Table 1). As far as the tumor cell suspensions were concerned, the second generation of the tumor cells showed highest capacity to incorporate 1-14C acetate into phospholipids and fatty acids and this ability showed a gradual well-marked decline in the case of phospholipids and a slight but definite decrease in the case of fatty acids as the third, fourth, fifth and sixth generations of tumor cells were used for the incorporation studies. But there were no such well marked changes in the rate of incorporation of 1-14C acetate into the cholesterol fraction.

Lee and co-worker in their study of the uptake of P32 by livers of ascites tumor-bearing mice (1962) have reported that the specific P32 activities of the individual phosphatides of the livers of tumor-bearing mice were greatly increased above those of the livers from normal mice. In the results reported here, a higher capacity to incorporate 1-14C acetate not only into the phospholipid fraction but also into the cholesterol and fatty acids fraction was observed. The high content of total phospholipids in the

TABLE III

DISTRIBUTION OF RADIOACTIVITY IN DIFFERENT TYPES OF FATTY ACIDS OF PHOSPHOLIPIDS

Percentage of recovery of added radioactive fatty acids from the silver nitrate-silicic acid column		% of different fatty acids present in the recovered sample			
		Saturated	Mono ethylenic	Di ethylenic	Tri ethylenic
NL	91.3	40.8	15.4	13.7	30.1
TL	90.6	80.5	6.2	7.5	5.8
TC	94.0	51.9	6.9	27.4	13.7

NL = Livers from normal mice; TL = Livers from tumor-bearing mice; TC = Tumor cells.

tumors as compared with the normal tissues, as reported by Lee *et al* may thus be due to the higher synthesis of phospholipids by the tumor tissues. The view that the total phospholipid content of tumor tissue is higher than that of the normal tissue is contrary to that of Figard and Greenberg (1962) who maintain that the total phospholipids present in all liver tumors are much less than that in normal liver.

The ratio of radioactivity in lecithin to cephalin did not show any appreciable change in the lipids from livers of normal and tumor-bearing mice. This ratio was 1:2 in the case of lipids from livers of normal mice and 1:1·6 in the case of lipids from livers of tumor-bearing mice, but has changed appreciably in the case of lipids from tumor cell suspensions to 1:7. In livers from normal and tumor-bearing mice, as well as in ascites tumor cells the incorporation of acetate 1-14C into the other phospholipid fractions were found to be negligible.

Examination of the results of analysis of the fatty acids methyl esters from the total phospholipids revealed that the phospholipids from normal livers had 41% of the total radioactivity of the phospholipids in the saturated fatty acids, 15%, 14% and 30% respectively in the mono, di and tri ethylenic fatty acids, whereas in the case of the livers from tumor-bearing mice, 80% activity was seen in saturated fatty-acids fraction and only 6%, 8% and 6% respectively in the mono di and tri ethylenic fatty acids. Thus a marked contrast in the incorporation of acetate 1-14C between the livers from normal and tumor-bearing mice was observable. One point of interest relating to the fatty acids composition of these phosphatides was concerned with the high proportion of 1-14C acetate incorporation into the saturated fatty acids by the livers from tumor-bearing mice and a sharp fall in the 1-14C acetate incorporation into the mono, di and tri ethylenic fatty acids by the same tissues. In the case of ascites tumor cells, 52% of the total activity of phospholipids was found to be present in saturated fatty acids, 7% in mono ethylenic fatty acids, 27% in diethylenic fatty acids and 14% in triethylenic fatty acids. It may be mentioned that no attempt was made to identify the various individual fatty acids in each group. The observations made here are not in agreement with those of Gray (1963) who has observed an absence of any marked difference in the composition of phospholipids from tumor cells and normal tissue.

SUMMARY

In a comparative study with six consecutive generations of ascites tumor cells, the liver slices of the tumor-bearing mice and that of normal mice, it was found that the livers of the tumor-bearing mice incorporated much more 1-14C acetate into the total phospholipids, fatty acids and cholesterol fractions when compared with normal liver slices or tumor cell suspension. Of the six generations of tumor cells, the second generation exhibited highest capacity to incorporate 1-14C acetate into phospholipids and fatty acids. Liver slices from normal and tumor-bearing mice incorporated 1-14C acetate almost in the same proportion into the lecithin and cephalin fractions but the tumor cells incorporated about seven times 1-14C acetate into cephalin fraction as compared to lecithin fraction.

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