THE CATABOLIC METABOLISM OF CHOLESTEROL IN STOMACH CANCER

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5 α-cholestan-3ß-ol (dihydrocholesterol) was discovered by Windaus and Ubrig (1), and available information has given rise to the impression that 5α-cholestan-3ß-ol is an end product of cholesterol metabolism and undergoes no further transformation (2).

Determination of 5 α-cholestan-3ß-ol has been reported by Schoenheimer et al. (3). "A derivative isotope dilution method for 5α-cholestan-3ß-ol" has recently reported (4).

Though many attempts have been made to show some correlation between malignant tumor and sterol, the problem is still far from being solved. Baumann et al. (5, 6) found that cholesterol when fed did not promote tumor growth, but, when applied locally in oil, might acceralate the development of cancer produced by such agents as ultraviolet light (7) or benzpyrene (6). The production of skin carcinoma in rats by ultra-violet light is preceeded by local concentration of cholesterol (8). Bergmann et al. (9), however, found that irradiated cholesterol did not act as a carcinogen. Kuroda and Chaikoff (4) reported that in tumorous adrenal gland of rats 5α-cholestan-3ß-ol is lower than normal, and cholesterol, too, distinguishedly decreased. They found the same phenomenon in patients of adrenal gland tumor with Cushing syndrome. The authors hope this investigation of 5α-cholestan-3ß-ol and cholesterol in gastric carcinama prove to be interesting for cancer researchers.

EXPERIMENT

Materials: Specimens of cancers of the stomach were obtained from gastrectomies at Okayama University Hospital. Table I shows cases of stomach cancer, the parts obtained and histological diagnosis.

Cancerous and non-cancerous tissues were separately taken from the same stomach which were

Table 1

Case NO	Age	Sex	Body weight	Histodiagnosis	Cancerous	Non-cancerous
					Tissues obtained from	
1	40	M	50 kg	Adenocarcinoma tubulare	cardia	pylorus
2	59	M	45	2	cardia	pylorus
3	44	M	48	〃	cardia	pylorus
4	48	W	48	〃	pylorus	cardia
5	54	M	45	Adenocarcinoma acmnosum muconodulare	pylorus	cardia
					pylorus	cardia
6	64	W	41.6	Adenocarcinoma solidum simplex scirrhosum	pylorus	cardia

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histologically examined. Stomach cancer tissue has much necrotic tissue, which was macroscopically removed as much as possible.

Non-cancerous tissues were taken from distant parts from the cancer; for instance, from the cardia in case of pyloric cancer. Fig. 1 shows a case of pyloric carcinoma.

Fig. 1 A Case of Pyloric Cancer

for cancerous tissue

for non-cancerous tissue

Acetic acid-1-C^{14} (5.0mc/m. mol.) was diluted with about 7 ml of unlabelled acetic anhydride. Cholesterol, arranged by Sionogi Research Laboratory, was purified (10^{-}, and it was regenerated as described in (11, 12). Its acetyl derivative was also prepared.

Extractiou of Sterol: Cancerous and non-cancerous tissues were weighed on a chemical balance and homogenized in a 3: 1 alcohol-ether mixture. The tissues were extracted twice by refluxing with 20 volune of the 3: 1 alcohol-ether solution, each time for one hour. The extracts were diluted so as to yield a 50% aqueous solution, which was then extracted three times with equal volume of petroleum ether. The combined petroleum ether extracts were washed with water and were dried over anhydrous sodium sulfate. After vacuum distillation of the petroleum ether, the residue was chromatographed on the silicic acid to separate the free sterol from the esterified by the procedure described in (13, 14). The sterol esters were hydrolysed with 10% potassium hydroxide in alcohol by heating one hour on the steam bath. The hydrolysate was diluted with water and extracted three times with petroleum ether. The combined petroleum ether extracts were washed and dried as described above and distilled with vacuum. The dried residue and the free sterol previously eluted from silicic acid chromatography were analysed for cholestanol and cholesterol.

Digitonin precipitation was preceeded of the method in (15), then digitonides were split with dry pyridine (16).

Derivative Isotope Dilution Method; Both the crystalline and non-crystalline sterol fractions were transferred to 8 X 90mm test tube, and dried completely in the Abderhalden' apparatus for 30 minutes. To each tube 50 micro-liter of acetic anhydride-1-C^{14} was added, and a small funnel containing a glass bead was placed in the mouth of the test tude. The tube was heated

in a paraffine bath maintained at 142—145 °C for 90 minutes and was then cooled. The content of each tube was transferred quantitatively 50.0 mg of 5α-cholestan-3ß-ol acetate and cholesteryl acetate and 5 ml of methanol. The methanol and chloroferm were removed by vacuum distillation. After vacuum distillation of the solvents, the process was repeated twice. The sterol residue was transferred to a small vial and dissolved in a chloroform solution containing about 0.g4 perbenzoic acid. The solution was kept at room temperature in the dark for two hours, and was then diluted with 20 ml of 4 : 1 ether-chloroform. * The solution was neutralized with 5 per cent sodium carbonate. After the ether-chloroform solution was washed with water, it was dried over anhydrous sodium sulfate, and distilled to dryness. The residue, dissolved in petroleum ether, was transferred on a 10 g column of aluminum oxide (Merck). The column was developed with 100 ml of petroleum ether containing 2 per cent benzene, and radioactive stanol was eluted with 300 ml of petroleum ether containig 10 per cent of benzene. The solvent was removed by vacuum distillation and the stanol acetate was recrystallized twice from acetonmethanol (1 : 1). Then 100ml of 20 per cent benzene in petroleum ether was eluted. At last, radioactive epoxide cholesteryl acetate was effluented with 300 ml of 100 per cent benzene. The solvent was distilled by vacuum, and then recrystallized twice from a methanol-water solution (1 : 1).

The radioactive stanol eluted by 10 per cent benzene in petroleum ether is 5α-cholestan-3ß-ol acetate and the purity * was estalbished by its melting point. Epoxide cholesteryl acetate is α, ß compounds and its melting point is at 110°C (softens at 86°C).

Determination of C^{14}-Radioaotivity: 30—40 mg of the C^{14}-5α-cholestan-3ß-ol acetate and 2—5 mg of epoxide cholesteryl acetate was dissolved in 15 ml toluene containing 1.5 mg of 1,4-bis2-(5-phenyloxazolyl)-benzene and 40 mg of 2,5-diphenyloxazol, and its C^{14} was assayed by liquid scintillation spectrometer, Packard Tri-Card Model 314a.

Calculation: The calculation was carried out by using the following equation:

$$\text{mg of DHC} = \frac{\text{(Mol. wt. DHC) X (Total DPM for Carrier)}}{\text{(Mol. wt. DHC-acetate) X (DPM for DHC-acetate Stand. /mg)}}$$

and

$$\text{mg of cholesterol} = \frac{\text{(Mol. wt. Epox-acetate) X (Mol. wt. . Chol.)X(Total DPM for Carrier)}}{\text{(Mol. wt, Chol. -acetate)X(Mol. wt. DHC-acetate) X (DPM for DHC-acetate Stand/mg)}}$$

DHC: dihydrocholesterol: 5α-cholesten-3ß-ol

Epex-acetate: epoxied cholesteryl acetate

Chol.: cholesterol

Chol, -acetate: cholesteryl acetate

DHC-acetate Stand.: 5α-cholestan-3ß-ol acetate Standard

This Standard was previously made with the same method by using 100 mg 5α-cholestan-3ß-ol being acetylated with 200 micro-liter of acetic anhydride-1-C^{14} as described in Derivative Isotope Dilution Method.

RESULTS AND DISCUSSION

Table II and Table III show 5α-cholestan-3ß-ol (DHC) and cholesterol in stomach cancer tissues. [In this procedure cholesteryl acetate changes to α, ß epoxide cholesteryl acetate.]

Table 11 Microgram of DHC in non-cancerous & cancerous tissues

Case No	Non-cancerous		Cancerous	
	Free	Ester	Free	Ester
1	0.076	0.142	0.025	0.155
2	0.089	0.293	0.026	0.076
3	0. 123	0.210	0.047	0.060
4	0.037	0.075	0.069	0.164
5	0.044	0.179	0.070	0.120
6	0.054	0.126	0.087	0.159

(unite; microgram öf 100 gm body weight)

Table 111 Microgram of Cholesterol in Non.cancerous & Cancerous Tissues

Case No	Non-cancerous		Cancerous	
	Free	Ester	Eree	Ester
1	0.173	0.536	0.556	0.933
2	0.193	1.024	0.213	1.172
3	0.437	0.866	0.204	0.720
4	0.091	1.237	0.399	1.357
5	0.095	1.574	0.299	1.017
6	0.156	1.656	0.279	0.975

(unit; microgram of 100 gm body weight)

The case number is the same as Table 1. Table 1V shows a ratio of 5α-cholestan-3ß-ol and cholesterol, and it does not mean the total sterol amount, but indicates the value of 5α-cholestan-3ß-ol and cholesterol.

Table 11 and Table 111 are shown by the unit of microgram per 100 g of body weight. The authors could not find from their statistics any definite evidence. This was due to, individual differences and differences on the stage of the cancer, and excision of the specimen at various surgical suages. More over, non-cancerous tissues excised from a cancerous environment is influenced by it.

Table 1V statistics indicate percentage, and influences of many conditional differences are smaller than in Table 11 and Table 111. Among the non-cancerous group the difference of the

Table 1V A Ratio of DHC & Cholesterol in Non-cancerour & Cancerous Tissues

Case No	Non-caucerous				Cancerous			
	Eree		Ester		Free		Ester	
	DHC	Cholesterol	DHC	Cholesterol	DHC	Cholesterol	DHC	Cholesterol
1	8.2	18.7	15.3	57.8	1.5	33.3	9.3	55.8
2	5.6	12.1	18.3	64.0	1.8	14.2	5.1	78.2
3	7.7	23.7	13.1	54.1	4.5	19.8	5.8	69.8
4	2.6	6.3	5.2	85.8	3.3	19.6	10.4	66.5
5	2.3	5.0	9.4	82.4	5.1	20.4	7.8	66.7
6	2.7	7.9	6.1	83.2	5·4	23.7	10.0	61.0

examined parts, the cardia and pylorus was found. Free 5α-cholestan-3ß-ol and cholesterol and esterified 5α-cholestan-3ß-ol of non-cancerous tissues of pylorus are higher than that of the cardia. Esterified cholesterol of pylorus are lower than that of the carida. The digesting movement of the pylorus may be influencing factor. It suggests that the pylorus has more active movement than the cardia, and its cholesterol metabolism is more active than the cardia.

Among cancerous tissues free 5α-cholestan-3ß-ol of the cardia is lower than the pylorus, and their mean value is lower than that of the non-cancerous cardia. Free 5α-cholestan-3ß-ol of pyloric cancer is lower than that of the non-cancerous pylorus. Free cholestrol of the cancerous group is higher than the non-cancerous. Esterified 5α-cholestan-3ß-ol and cholesterol of cardia cancer are lower than the nou-cancerous, but the difference is not marked from the non-cancerous tissues. Esterified 5α-cholestan-3ß-ol and cholesterol of pyloric cancer is lower than the non-cancerous cardia, but is observed to be almost the same as the non-cancerous pylorus.

It is interesting that among the cancerous group the ratio of free cholesterol is higher than that of the non-cancerous. This suggests that while the authors could hardly find any definite evidence in Table 11 and Table 111, esterified cholesterol might be mainly used for catabolic metadolism in cancerous tissues. Esterified 5α-cholestan-3ß-ol and cholesterol have smaller ratios in the cancerous tissues than in the non-cancerous. These phenomena are similiar to Kuroda and Chaikoff's report about adrenal gland (*4*), that in tumorous adrenal gland esterified 5α-cholestan-3ß-ol and cholesterol are smaller than(normal).

In the experimental process, it has been noticed tnat the non-cancerons tissue extract solution has a yellowish colour, and the extract solution of the cancerous tissues was colourless compared with the non-cancerous.

The smaller amount of esterified cholesterol in cancerous tissues means that the cholesterol metabolism is more active than in non-cancerous tissues; or, the cancer patients had avitaminosis of Vitamin A and the synthesis of cholesterol from mevalonic acid has become decreased. The yellowish colour of the tissue extract solution may be an indication of this fact. It suggests that the amounts of carotinoid pigment or Ubiquinone (Co Q) smaller in cancerous tissues.

The smaller amount of esterified 5α-cholestan-3ß-ol in cancerous tisses means that, if 5α-cholestan-3ß-ol is the end product of cholesterol metabolism in tissus the reaction process from cholesterol to cholest4-en-3one is damaged and activity of Δ^4dehydrogenase decreased, the reaction process of cholest5-en-3ß-ol being also damages. If 5α.cholestan-3ß-ol is not the end product of cholesterol metabolism, further process from 5α-cholestan-3ß-ol to bile acid or pregan like substance may have occured.

These phenomena must be studied by enzymatic method in future.

SUMMARY

1. The ratios of free 5α-cholestan-3ß-ol and cholesterol and esterified 5α-cholestan-3ß-ol are higher in the pylorus than in the cardia.

2. Esterified cholesterol is higher in the cardia than in the pylorus.

3. Among stomach cancer tissues free cholesterol is higher than the non-cancerous.

4. Eaterified 5α-cholestan-3ß-ol and cholesterol are lower in the cancerous tissues than in the non-cancerous,

This suggests that carotinoid pigment or Co-enzyme Q and Δ^4 dehydrogenase are decreased in cancerous tissues, or further process from 5α-cholestan-3ß-ol to bile acid or pregnan like substance is made in cancerous tissues.

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