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Abstract

1. The ratios of free 5α-cholestan-3β-ol and cholesterol and esterified 5α-cholestan-3β-ol were higher in pylorus than in cardia. 2. Esterified cholesterol level was higher in cardia than in pylorus. 3. Among the stomach cancer tiues examined free cholesterol level was higher than in the non-cancerous. 4. Esterified 5α-cholestan-3β-o1 and cholesterol levels were lower in the cancerous tiues than in the non cancerous.

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CATABOLIC METABOLISM OF CHOLESTEROL IN STOMACH CANCER

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

Determination of 5α-cholestan-3β-ol has been reported by SHOENHEIMER *et al.*[3] and there is also a recent report on " a derivative isotope dilution method for 5α-cholestan-3β-ol ".

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 accelarate the development of cancer produced by such agents as ultraviolet light[7] or benzpyrene[6]. The production of skin carcinoma in rats by ultraviolet light is preceded by local concentration of cholesterol[8]. BERGMANN and his associates[9], however, found that irradiated cholesterol did not act as a carcinogen. KURODA *et al.*[4] reported that in tumorous adrenal gland of rats 5α-cholestan-3β-ol was lower than in normal, and cholesterol was also distinctly decreased. The same phenomenon was observed in adrenal gland tumor of patients with Cushing's syndrome. This investigation was undertaken to clarify the catabolism of cholesterol in gastric carcinoma.

MATERIALS AND METHODS

Materials: Specimens of stomach were obtained at Okayama University Hospital. Table 1 shows the cases of stomach cancer, the parts of specimens obtained, and histological diagnosis.

Cancerous and non-cancerous tissues were taken separately from the same stomach, which were histologically examined. Stomach cancer tissue had 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 the case of pyloric cancer. Fig. 1 shows a case of pyloric carcinoma.

Acetic acid-C^{14} (5.0 mc/m mol) was diluted with 7 ml of unlabelled acetic anhydride. Cholesterol was purified before the use and 5α-cholestan-3β-ol was donated by Shionogi Research Laboratory. With these compounds acetyl derivatives were also prepared.

Extraction of sterol : Cancerous and non-cancerous tissues were weighed on a chemical balance and homogenized in the ethanol-ethyl ether mixture (3 : 1, v/v). The tissues were extracted twice by refluxing with 20 volumes of the ethanol-ethyl ether solution for one hour each. After condensing the combined ethanol-ethyl ether solution to half volume *in vacuo*, an equal amount of water was added and then extracted three times with equal volume of petroleum ether. The combined petroleum ether extract was washed with water and dried over anhydrous sulfate. After evaporation of the petroleum ether *in vacuo*, the residue was dissolved with a small amount of n-hexane and chromatographed on silicic acid to separate the free sterol from the esterified one by the procedure described by HANAHAN *et al.*[10,11]. The sterol ester was hydrolyzed with 10 per cent KOH-ethanol for one hour at 85°C on a steam bath. The hydrolysate was extracted three times with petroleum ether. The combined petroleum ether extract was washed with water and evaporated *in vacuo* as described above. The dried residue and free sterol previously eluted from silicic acid chromatography were analysed for 5α-cholestan-3β-ol and cholesterol.

Digitonin precipitation was carried out by the method of SCHOENHEIMER *et al.*[12], and then digitonide was split with dry pyridine[13].

Derivative isotope dilution method : Both the crystalline and non-crystalline sterol fractions were transferred to the test tube (8 × 90 mm), and dried completely in Abderhalden's apparatus for 80 min. To each tube 50 micro liters of acetic anhydride-1-C^{14} were added, and a small funnel holding a glass bead on its hole was placed in the mouth of the test tube. The test tube was heated on a paraffine bath maintained at 142—145°C for 90 min. and was then cooled. The content of each tube was transferred with chloroform to the 100 ml round-bottom flask containing 50 mg of 5α-cholestan-3β-ol acetate and cholesteryl acetate, and 5 ml of methanol were added to the flask. The chloroform-methanol mixture was removed by vacuum distillation. The residue was dissolved with a small amount of chloroform, and then 5 ml of methanol was added and evaporated. This procedure was repeated twice. The sterol residue was transferred to a small vial and dissolved in a 10 ml of chloroform solution containing about 0.4 g of perbenzoic acid. The solution was kept at room temperature in the dark for two hours, and was then washed with 5 per cent Na_2CO_3 to remove the excess perbenzoic acid. The chloroform solution was diluted with 20 ml of

ethyl ether, then dried *in vacuo*. The residue, dissolved in petroleum ether, was transferred on a 10 g column of aluminium oxide (Merck) which was activated at 180°C overnight. The column was developed with 100 ml of petroleum ether and the eluate was discarded. Radioactive stanol was eluted with 300 ml of petroleum ether containing 10 per cent of benzene. The eluate was evaporated to dryness and recrystallized twice with acetone-methanol (1 : 1). Then 100 ml of 20 per cent benzene in petroleum ether were eluted and discarded. Finally, radioactive epoxide cholesteryl acetate was eluted with 300 ml of 100 per cent benzene. The eluate was distilled *in vacuo*, and then the residue was recrystallized twice with methanol-water (1 : 1).

The redioactive stanol eluted by 10 per cent benzene in petroleum ether was 5α-cholestan-3β-ol acetate, and the purity was checked by determining its melting point. Epoxide cholesteryl acetate was an α and β compound and its melting point was at 110°C (softened at 86°C).

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

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

$$\text{mg of DHC} = \frac{(\text{Mol. wt. DHC}) \times (\text{Total cpm for Carrier})}{(\text{Mol. wt. DHC-Ac}) \times (\text{cpm for DHC-Ac-Stand./mg})}$$

and

$$\text{mg of cholesterol} = \frac{(\text{Mol. wt. Epox-Ac}) \times (\text{Mol. wt. Chol.}) \times (\text{Total cpm for Carrier})}{(\text{Mol. wt. Chol.-Ac}) \times (\text{Mol. wt. DHC-Ac}) \times (\text{cpm for DHC-Ac-Stand./mg})}$$

DHC : dihydrocholesterol : 5α-cholestan-3β-ol

Epox-Ac : epoxide cholesteryl acetate

Chol. : cholesterol

Chol.-Ac : cholesteryl acetate

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

This Standard was previously prepared by the same method as reported by KURODA[4], using 100 mg of 5α-cholestan-3β-ol, acetylated with 200 microliters of acetic anhydride-1-C^{14}.

RESULTS AND DISCUSSION

Since the work of SCHOENHEIMER in 1930, 5α-cholestan-3β-ol was studied from the standpoint of metabolism and function in animals. Many quantitative

methods for determining this substance were presented in the past, but these methods were difficult to reproduce with limited materials.

KURODA *et al.* reported a useful method for microdetermination[4], and the attractive point of this method was the accuracy in the determination of 5α-cholestan-3β-ol, using an isotope dilution method and polarizing cholesterol by epoxidation to separate 5α-cholestan-3β-ol and cholesterol with column chromatography.

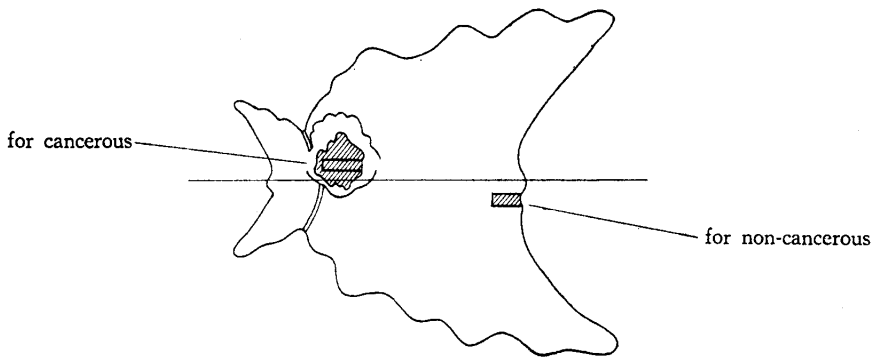

Fig. 1 A Case of Pyloric Cancer

Table 1 Cases of Stomach Cancer

Case	Age	Sex	Body weight	Histodiagnosis	Cancerous Tissues obtained from	Non-cancerous Tissues obtained from
1	40	M	50 kg	Adenocarcinoma tubulare	Cardia	Pylorus
2	59	M	45	Adenocarcinoma tubulare	Cardia	Pylorus
3	44	M	48	Adenocarcinoma tubulare	Cardia	Pylorus
4	48	F	48	Adenocarcinoma tubulare	Pylorus	Cardia
5	54	M	45	Adenocarcinoma acinosum muconodulare	Pylorus	Cardia
6	64	F	42	Adenocarcinoma solidum simplex scirrhosum	Pylorus	Cardia

Tables 2 and 3 show 5α-cholestan-3β-ol (DHC) and cholesterol in stomach cancer tissues. The case number is the same as in Table 1. Table 4 shows the ratio of 5α-cholestan-3β-ol to cholesterol, and does not mean the absolute sterol amount.

The data in Tables 2 and 3 are shown by the unit of microgram per 100 g of tissue weight. These data did not indicate any characteristic results for stomach

Table 2 5α-Cholestan-3β-ol
in Non-cancerous and Cancerous Tissues in Stomach

Case	Non-cancerous		Cancerous	
	Free	Ester	Free	Ester
1	0.076	0.142	0.025	0.155
2	0.086	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

(unit: microgram per 100 g tissue)

The values shown in [ ] were obtained from pylorus and in [ ] from cardia.

Table 3 Cholesterol
in Non-cancerous and Cancerous Tissues in Stomach

Case	Non-cancerous		Cancerous	
	Free	Ester	Free	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 per 100 g tissue)

The values shown in [ ] were obtained from pylorus and in [ ] from cardia.

cancer. This was probably due to the individual differences of patients. Moreover, it is presumed that non-cancerous tissue obtained from the same specimen was influenced by cancerous environments.

The data in Table 4 are represented by percentage ratio, and influences of many conditional differences were probably smaller than in Table 2 and Table 3. Among the non-cancerous tissues, the differences of values were observed between cardia and pylorus. In pylorus, the percentage ratio of free 5α-cholestan-

Table 4 The Ratio of 5α-Cholestan-3β-ol and Cholesterol in Non-cancerous and Cancerous Tissues of Stomach

Case	Non-cancerous				Cancerous			
	Free		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

(DHC+Cholesterol=100 per cent)
Other steroids are omitted in this table.
The values shown in [ ] were obtained from pylorus and in [ ] from cardia.

3β-ol and cholesterol, and esterified 5α-cholestan-3β-ol was higher than that in cardia, but esterified cholesterol in pylorus was lower than that in cardia. It suggests that the digesting movement of pylorus might be a potent factor, and pylorus had more active movement than cardia. Also the cholesterol metabolism in pylorus was more active than in cardia.

Among the cancerous tissues examined free 5α-cholestan-3β-ol in cardia was lower than in pylorus. Free 5α-cholestan-3β-ol of pyloric cancer was lower than that of the non-cancerous pylorus. Free cholesterol of the cancerous group was higher than that of the non-cancerous. Esterified 5α-cholestan-3β-ol of cardiac cancer was lower than the non-cancerous. That of pyloric cancer was lower than the non-cancerous cardia, but was observed to be almost the same as the non-cancerous pylorus.

It is interesting to note that among the cancerous tissues the ratio of free cholesterol was higher than that of the non-cancerous, and esterified 5α-cholestan-3β-ol and cholesterol had smaller ratios in the cancerous tissues than in the non-cancerous tissues, while any definite indication could hardly be discerned from Table 2 and Table 3. It suggests that esterified cholesterol might be mainly used for catabolic metabolism in cancerous tissues. These phenomena were similiar to the report of KURODA *et al.* about adrenal gland[4].

KELLER suggested an altered sterol metabolism in adrenal tumor tissue[14]. HAVEN *et al.*[15] and BEGG[16] reported that rats bearing Walker-carcinoma and

Jensen sarcoma have adrenal hyperplasia and lower adrenal cholesterol content than that of normal. GORE *et al*[17]. found that the rat ascites tumor and mouse Ehrlich ascites carcinoma are unable to utilize acetate for the sterol synthesis.

From the fact that a smaller amount of esterified cholesterol was contained in cancerous tissue, it was considered that the cholesterol metabolism was more active in the cancerous tissues than in the non-cancerous tissues.

SUMMARY

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

2. Esterified cholesterol level was higher in cardia than in pylorus.

3. Among the stomach cancer tissues examined free cholesterol level was higher than in the non-cancerous.

4. Esterified 5α-cholestan-3β-ol and cholesterol levels were lower in the cancerous tissues than in the non-cancerous.

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