STUDIES ON CALCIUM, SODIUM, AND POTASSIUM CONTENT OF THREE STRAINS OF ASCITES HEPATOMA, WITH SPECIAL REGARD TO THEIR BIOLOGICAL SIGNIFICANCE

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That Ca is decreased and K increased in cancerous tissues as compared to the corresponding normal ones, has hitherto been reported by many workers.[1~10] DeLong *et al.*[5] reported that the diminution in Ca content is peculiar to cancer and responsible for the decreased mutual adhesiveness of cancer cells. They also reported that the increased K content is merely one of the expressions of cellular multiplication, since increase in K is found in actively growing tissues, whether cancerous or noncancerous. The lowered Na content of cancers was also observed by them. However, all these studies were performed with cancer tissues containing stroma. The present author attempted to determine the content of Ca, Na, and K in cancer cells themselves, using ascites hepatoma and to make clear the biological significance of these cations.

MATERIALS AND METHODS

Tumor-materials—Three strains of ascites hepatoma, AH 130, AH 602, and AH 7974, established by Yoshida,[11] were used. Wistar strain rats weighing about 100 gm were employed. Transplantation of the ascites hepatoma was performed intraperitoneally with 0.5 ml of undiluted tumor ascites. Further, in order to study the growth rate of these tumors, another group of animals received subcutaneous inoculations of 0.1 and 0.3 ml of undiluted tumor ascites. The ascites hepatoma inoculated subcutaneously was prone to show regression. Therefore, the tumor was weighed on the 7th day after inoculation.

Determination of Ca, Na, and K contents of tumor cells—The contents of these cations in the ascites hapatoma cells were determined according to the method described by DeLong *et al.*[5] About 2 ml of tumor ascites of each strain in a state of nearly pure culture was withdrawn from the peritoneal cavity by means of fine glass pipettes. It was washed with 0.25 M cold sucrose solution and centrifuged. Tumor cells thus washed were dried to constant weight at 105° C. and then ashed in crucibles at 550° C in a muffle furnace. The ash was dissolved in 0.1 N HCL. Ca, Na, and K were estimated at 554

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mμ, 589 mμ and 768 mμ irrespectively, using the Hitachi flame photometer. All results were calculated on the basis of dry weights.

RESULTS

Ca, Na and K contents of three strains of the ascites hepatoma are summarized in Table 1. The difference in average contents of these cations in the three strains was tested statistically and the results are also shown in Table 1. From these results, the order of increasing Ca content in tumor cells of these three strains was found to be AH 130, AH 7974 and AH 602, *i.e.*, the Ca content of AH 130 was lowest and that of AH 602 highest. AH 602 was highest also in Na content, but there was no significant difference in the Na contents of AH 130 and AH 7974. Regarding K, the content in AH 7974 was greater than in AH 130, but the difference in K content between AH 602 and AH 7974 or AH 130 was not significant statistically. Results concerning the growth rate of these tumor are shown in Table 2. The growth rate of AH 130 was most rapid and of AH 602 most slow. AH 7974 was moderate. These results closely agreed with the median survival time of animals which received intraperitoneal inoculations of these tumors.[12]

TABLE 1. Calcium, Sodium, and Potassium Contents of three Strains of Ascites Hepatoma

Tumor strain	Number of animals	Number of specimens	Average content of Ca, Na and K Mg. per hundred mg based on dry weight		
			Ca Mean±S.E.	Na Mean±S.E.	K Mean±S.E.
AH 130	7	18	0.0386±0.0019	0.321±0.027	1.119±0.124
AH 7974	6	21	0.0474±0.0026	0.316±0.034	1.466±0.034
AH 602	5	17	0.0752±0.0092	0.531±0.063	1.380±0.065
Probability					
AH 130 vs AH 7974			P<0.001	P>0.05	P<0.01
AH 130 vs AH 602			P<0.001	P<0.01	P>0.05
AH 7974 vs AH 602			P<0.01	P<0.01	P>0.05

TABLE 2. Growth Rate of Ascites Hepatoma Inoculated Subcutaneously

Inoculation dose of tumor (ml)	Average tumor weight in gm. and variation extremes*		
	AH 130	AH 7974	AH 602
0.1	0.60(0.40–0.90)	0.52(0.40–0.61)	0.23(0.07–0.35)
0.3	0.74(0.40–1.20)	0.60(0.31–0.90)	0.37(0.06–1.00)

* Averages of 5 rats on the 7th day after inoculation are indicated.

DISCUSSION

As reported by Yoshida[11] and Kurata,[13] the ascites hepatoma used in this study is not a free-cell tumor in the strict sense of the word, but the tumor

cells, being epithelial in nature, make cell associations, or "islands", suspended in ascitic fluid, and individually isolated cells are also present. The number of these isolated cells differs depending upon the hepatoma strain. They are most abundant in strain AH 130. Isolated cells are observed also in strain AH 7974, but are usually rare in AH 602. At the same time, in the tumor strain containing the more separated free-tumor cells, the size of "islands" was the smaller.

Consequently, from the results obtained in this study it is evident that there is a reverse relationship between the content of Ca of tumor cells and the number of single isolated tumor cells in the ascites hepatomas. Coman and his coworkers[5)14)] confirmed that the Ca content is decreased in cancerous tissues as compared to the corresponding normal ones and that the diminution in Ca content is responsible for decreased mutual adhesiveness of cancer cells. The present study performed with cancer cells per se of ascites hepatoma seems to support more concretely their conclusions. As to cell motility, the author[12)] has reported in a previous paper that AH 130 showed the most active motility, AH 7974 moderate, and AH 602 least. Accordingly, it can be inferred that there is an intimate relationship between mutual adhesiveness of tumor cells and their motility, as was reported by Coman.[14)] DeLong *et al.*[5)] reported that the Na content is decreased in cancer when compared with normal tissues. The present results also showed that Na content of the tumor strain containing the largest amount of Ca was greater than in others. Regarding the K content, it has been reported that increase in K is found in actively growing tissues, whether cancerous or noncancerous.[5)] According to our results, however, it was difficult to conclude that the more rapidly growing tumor strain shows a greater content of K. A clear difference was not observed in K content among three strains, though it was observed in the growth rate. Furthermore, the K content in the most rapidly growing strain, AH 130, was less than in a more slowly growing one, AH 7974.

SUMMARY

Calcium, sodium, and potassium contents of tumor cells in three strains of ascites hepatoma, AH 130, AH 7974 and AH 602, were determined using the flame photometer. The order of increasing content of calcium in these three strains was AH 130, AH 7974 and AH 602. This fact supported the view that diminution in calcium content is responsible for decreased mutual adhesiveness of cancer cells. Further, the sodium content of the tumor strain which contains calcium most abundantly was found to be also greater than in the others. Regarding the potassium content, a distinct difference was not observed, though it was in growth rate.

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