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bearing animals I. Distribution of  
mucopolysacoharides in the organellae of liver  
in cancer (Hepatoma AH 130) bearing animals

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Studies on the organelles of liver of cancer bearing animals I. Distribution of mucopolysaccharides in the organelles of liver in cancer (Hepatoma AH 130) bearing animals\*

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**Abstract**

For the purpose to reveal whether or not the liver and the cell organelles are responsible for the abnormal metabolism of polysaccharides found in cancer bearing individuals, the author analyzed the liver and ascites with tumor cells of AH 130 hepatoma bearing rats biochemically with some histochemical observations. A quantitative increase in polysaccharides accompanied by the production of unusual polysaccharides is found in the supernatant of liver from cancer bearing rats, but not from mitochondrial or microsomal fractions. Tumor cells themselves and ascites fluid do not contain the abnormal polysaccharides found in the liver supernatant.

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## STUDIES ON THE ORGANELLAE OF LIVER OF CANCER BEARING ANIMALS

### I. DISTRIBUTION OF MUCOPOLYSACCHARIDES IN THE ORGANELLAE OF LIVER IN CANCER (AH 130 HEPATOMA) BEARING ANIMALS

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At present it is generally believed that in the cancer bearing individuals the metabolism of polysaccharides is severely distorted from the normal pathway. The KIK factor, which has been isolated from the gastric juice of the patients with stomach cancer by KOZAWA and IWATSURU<sup>1,2</sup>, et al. and identified as an anemia inducing factor, is essentially a polysaccharide composed of galactose, xylose, etc., as revealed by the recent works of MASAMUNE<sup>3,4,5</sup> and his collaborators. The reaction, inducing anemia in rabbit by injection of this factor, is now one of the common test for the early diagnosis of cancer bearing patients in Japan. MASAMUNE suggested that in the cancer bearing individuals the polysaccharide having the specific biological characteristics can be produced by the altered carbohydrate metabolism. It is supposed that the essential source of such abnormal polysaccharides might be the cancer cells themselves, but there is also a possibility that the connective tissue or the liver, which is especially rich in polysaccharides, is directly responsible for the synthesis of polysaccharides by their aberrant function resulting from the altered metabolism in the cancer bearing individual.

SEIBERT<sup>6,7</sup> showed that polysaccharides in serum are increased by the toxic damage of the liver cell as in the case of CCl<sub>4</sub>-or chloroform intoxication, suggesting the hepatic origin of the serum polysaccharides.

With the purpose to confirm the main organs responsible for the abnormal metabolism of polysaccharides in cancer bearing animals the author carried out several experiments on AH 130 hepatoma bearing rats. In the present paper the distribution of polysaccharides in the fractions of microsomes, mitochondria and supernatant obtained from the liver and tumors are reported.

MATERIALS AND METHODS

Ninety rats fed on oat, corn and cabbage served as test animals. These animals were divided into two groups. The first group consisting of 60 animals were inoculated with AH 130 hepatoma cells intraperitoneally. Two weeks after the inoculation a drop of ascites from each animal was taken and cytological tests were carried out on the smeared and dried specimens obtained with Giemsa. After confirming the full growth of tumor cells these animals were sacrificed by decapitation after being kept in starvation for 24 hours and ascites was collected for the chemical analysis. Small pieces of the liver of each animal were taken for histological test and the remaining part of the liver was homogenized immediately for the chemical analysis. The second group consisting of 30 pairs of the control, were sacrificed simultaneously after starvation and small pieces and homogenates of livers were prepared in the same manner as in the case of experimental animals. The homogenization of each material was done in a homogenizer supplemented with 10 volumes of 0.25 M sucrose solution kept at 0°C. These were centrifuged 2000 r. p. m. at 0°C for 10 minutes, respectively and the supernatants were obtained by removing precipitated nuclei and cell debris. The supernatant was superimposed on 0.35 M sucrose in a test tube and centrifuged at 10,000 r. p. m. at 0°C for 20 minutes and mitochondrial fractions were obtained as the precipitate. Supernatants were further centrifuged at 30,000 r. p. m. for 60 minutes at 0°C and microsomal fractions were obtained as the precipitate. These fractions of mitochondria and microsomes as well as cell supernatants were dialysed in running water for about 24 hours kept at 4°C. All the materials were frozen in liquid air bath and frozen-dried at about -20°C and kept in a cold room at 0°C until the chemical analysis.

The tissue stick from each liver for the histological observations was cut into two parts respectively. The one was fixed in 99 % ethanol and the other in 10 % formol. These were dehydrated, embedded in paraffin, sectioned by the routine method. The sections fixed with formol were stained with hematoxyline eosin for general observation and those with PAS stain for polysaccharides. The sections from those fixed with ethanol were stained with Best carmine for glycogen detection.

Qualitative and some quantitative chemical analysis for hexosamine, hexose, pentose, methylpentose and hexuronic acid were carried out in each fraction from the ascites tumor and the liver by the methods described in the following.

Fifty mg of each dried fraction from the liver or hepatoma ascites is hydrolyzed by adding 6 cc of 1N H<sub>2</sub>SO<sub>4</sub> or 4N HCl solution in boiling water bath for 20 hours. The pH of the supernatants is adjusted to 5.0 to 6.0 with 1N Ba (OH)<sub>2</sub> solution in the case of the hydrolysate of H<sub>2</sub>SO<sub>4</sub> and with 4N NaOH

in the case hydrolyzed by HCl. The solutions are concentrated to less than 0.5 cc at 30° to 40°C in vacuum. The concentrate is adjusted exactly to 2 cc by addition of distilled water. The solution is transferred to a column (0.9 × 10 cm) containing Dowex 50 (200 to 300 meshes). After washing with 50 cc distilled water, amino acids\* and amino sugars are eluted with of about 50 cc 1N HCl, until eluate gives no ninhydrin reaction. The sugars, hexose, pentose, hexuronic acid, etc., are then eluated with distilled water until the Molish reaction of the eluates becomes negative. The eluates thus obtained are concentrated to less than 0.5 cc at low temperature of about 40°C in vacuum and adjusted to 5 cc by adding distilled water in both cases.

One cc each of these adjusted solutions is used for the chemical analysis of amino sugars and amino acids. The quantitative estimation of amino sugar is carried out by Elson-Morgan's method at 560 m $\mu$  in each sample. As the standard solution 0.0131 % glucosamine solution is used. For the quantitative analysis of hexose the thionalid reagent is used. One cc of the water eluates is taken into a test tube and added with 3 cc of the diluted H<sub>2</sub>SO<sub>4</sub>, which is prepared by mixing 45 volumes of conc. H<sub>2</sub>SO<sub>4</sub> with 10 volumes of distilled water. After cooling the tubes with tap water to about 15°C, 0.3 cc of thionalid reagent (10 mg of thionalid in 30 cc of conc. H<sub>2</sub>SO<sub>4</sub>) is added and the tubes are heated in boiling water bath for 10 minutes and then cooled in ice water.

In treatment by the above reagent, glucose gives a pale pink color, but galactose and mannose give a light orange color, and methyl pentose, pentose, hexuronic acid and hexosamine remain colorless. Using the absorption band at 380 m $\mu$ , the total quantity of the hexoses is estimated by spectrophotometer.

The cysteine-HCl reaction is applied for the estimation of methylpentose and the thionalid method for the pentose. In the latter case 3 cc of conc. H<sub>2</sub>SO<sub>4</sub> diluted with 7.5 volumes of water is added before the addition of thionalid reagent and reacted in boiling water bath for 20 minutes. For the estimation of hexuronic acid, carboxyl reaction is applied and estimated at 530 m $\mu$ .

The qualitative analysis has been carried out on hexose prepared from the liver and hepatoma ascites by one dimensional paperchromatography; 0.006 cc each of the water eluates is spotted on Toyo filter paper No. 50 and hexose is developed with pyridin-butanol-water for 24 hours. As standard solution, 1 % solution of galactose, mannose, L-arabinose and xylose are used. Color development is made by spraying with phthalic acid aniline. Each sugar is distinguished by brown spots appearing separately.

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\* Distribution and qualitative analysis of amino acids in organellae are reported in the second paper.

## RESULTS

Generally, the livers of tumor bearing animals, all used for chemical analysis, showed some histological and histochemical changes. That is, in the preparate stained with hematoxylin-eosin the livers of the animals having hepatoma ascites showed the localized infiltration of small round cells in Glisson's capsule and slight changes of parenchymal cells with some atrophy and vacuolation. PAS staining proved an increased intensity in reaction appearing diffusely in liver parenchymal cells of tumor bearing animals and tumor cells comparing to that of normal liver. Glycogen as revealed by carmine staining was markedly reduced both in the liver parenchymal cells of tumor bearing and non-tumor bearing animals compared with that of the animals undergone no starvation.

The hexosamine content: Chemical analysis of hexosamine revealed that non-permeable component is contained in supernatant, microsomal and mitochondrial fractions, all from the livers of normal and tumor bearing animals and from the tumor ascites. Of the quantitative analysis, the mitochondria and the microsome of AH 130 cells and the liver of AH 130 bearing animal gave somewhat higher values than those from normal rat liver, the highest value in the liver of tumor bearing animals (Table 1), but no noticeable difference between the supernatants from them.

Table 1. Hexosamine contents of tumor.

AH 130 cells	CELL. SPN	1.21%
	MICROSOMES	1.01
	MITOCHONDRIA	0.85
AH 130 BEARING RATS LIVERS	CELL. SPN	1.91
	MICROSOMES	1.70
	MITOCHONDRIA	1.20
NORMAL LIVERS	CELL. SPN	1.22
	MICROSOMES	0.69
	MITOCHONDRIA	0.49

The hexose content:

Hexose is detected only in the supernatant in all cases, excepting the case of AH 130 tumor cells whose mitochondria contain some hexose. The striking fact is that the cell supernatant of the liver from the AH 130 bearing animal is quite rich in hexose, showing the value ten times as high as that of the supernatant of normal rat liver. However, the supernatants of tumor cell itself give

a rather low value, though 3 times as high as that of normal liver (Tables 2, 3).

The pentose content:

Pentose measured by cysteine-H<sub>2</sub>SO<sub>4</sub> reaction shows no quantitative difference between AH 130 tumor and livers of tumor bearing and normal animals, 2-3 per cent both in microsomal and mitochondrial fractions in all cases.

Table 2. Extinction of Hexose.

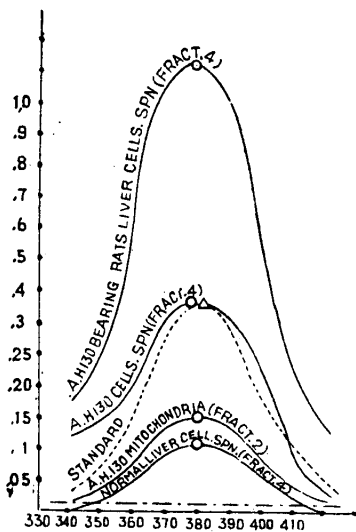


Table 3. Hexose contents.

AH 130 CELLS	CELL. SPN	9.8%
	MICROSOMES	0
	MITOCHONDRIA	4.4
	NUCLEUS	/
AH 130 BEARING RATS LIVERS	CELL. SPN	35.3
	MICROSOMES	0
	MITOCHONDRIA	0
	NUCLEUS	/
NORMAL LIVERS	CELL. SPN	3.3
	MICROSOMES	0
	MITOCHONDRIA	0
	NUCLEUS	/

The acid polysaccharides content:

Both methylpentose and hexuronic acid are not detected in any fractions from all the series of materials so long as the methods mentioned above are applied.

As already mentioned a quantity of non-dialyzable hexose has been detected in the supernatant from AH 130 cells and especially a considerable quantity in the liver cells of tumor bearing animals. In the next experiment the author have carried out qualitative analyses of the components by paperchromatography. Each fractions from AH 130 cells, the livers of tumor bearing and normal animals, dialyzed and hydrolysed, are extended by the method previously mentioned. The results are presented in Table 4. In the microsomal fractions only ribose is detected in all the series tested. In the mitochondrial fractions from normal liver, fucose, mannose and galactose have been detected but no ribose, whereas mitochondria from the liver of cancer bearing animal contain ribose but lack in normal components such as fucose, mannose and galactose.

The most interesting findings have been obtained with the supernatants from the livers of tumor bearing animals. The supernatants from the normal liver contain glucose and a trace of ribose and xylose, and one unidentified

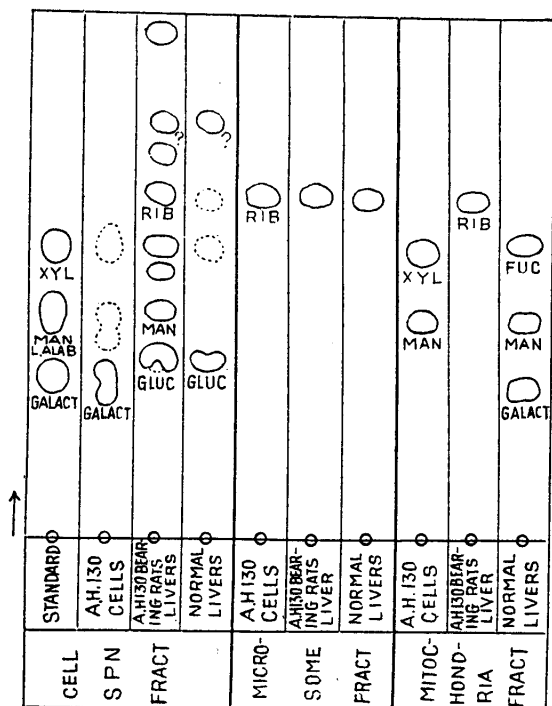


Table 4. PYRIDIN-BUTANOL-WATER

Toyo filter paper

No. 50.

GALACT=GALACTOSE.

MAN=MANNOSE.

L. ALAB=L. ALABINOSE.

XYL=XYLOSE.

GLUC=GLUCOSE.

RIB=RIBOSE.

FUC=FUCOSE.

component. All these components have been detected in the supernatant from the liver of tumor bearing animals, too, but rather intense in each reaction. Apart from these normal components a number of unknown substances have been contained, which give the pink color reaction diffusely as well as brown spots in all of them.

## DISCUSSION

Many observations in the past point toward severe disturbances in the polysaccharide metabolism in cancer bearing individuals. In the analytical study on blood sera from cancer patients, an increase in hexosamine has been reported by I. NILSSON (1937)<sup>6</sup>, increase in glucosamine by M. R. SCHETLER (1947)<sup>9</sup>, aminopolysaccharides by H. BERGSTERMANN (1955)<sup>10</sup> and glycoprotein by F. B. SEIBERT (1948)<sup>6,7</sup>. Among others, an excellent summary of R. J. WINZLER (1944-1948)<sup>11</sup> is available on this problem.

Concerning the source of these polysaccharides that increase in the sera of cancer patients, I. WERNER (1949)<sup>12</sup>, and Y. SHIOKAWA (1957)<sup>13</sup> noted its source to the liver and mesenchymal tissues actually having tumors.

H. R. CATCHPOLE (1950)<sup>14</sup> reported that the depolymerized polysaccharides in connective tissues will actually be the main source of the increased serum polysaccharides. I. BANGA (1957)<sup>15</sup> successfully demonstrated the increased ac-



tivity of mucoproteinase in the sera of cancer patients, which may be responsible for the depolymerization of polysaccharides. All these works show that some quantitative changes in the polysaccharide metabolism should exist in cancer disease.

H. MASAMUNE was the first to elucidate that the qualitative changes in the polysaccharide metabolism also exist in cancer bearing individuals. H. MASAMUNA and S. HAKOMORI<sup>16</sup> found unusual polysaccharides in the urine of patients of stomach cancer and proposed the name, K-mucopeptides, for this substance, illustrating its chemical components. The author has been interested in these polysaccharides and tried to reveal what cells or organellae are responsible for the production of such abnormal polysaccharides produced in cancer bearing individuals. As demonstrated above the unidentified polysaccharides, on the whole, have been proved to be contained in the liver supernatant from the rats bearing AH 130 hepatoma cells, but not from that of tumor cells and ascites cells. No mitochondrial and microsomal fractions from liver or tumor cells contain such abnormal polysaccharides. Quantitative increase in polysaccharides in the liver supernatant from the cancer bearing animals has also been demonstrated, about 10 times as high as that of normal liver and 3 times as that of tumor cells. T. TAKEUCHI (1957)<sup>17</sup> demonstrated histochemically phospholylase and transflucosidase in liver parenchymal cells, asserting that mitochondria will be the main organellae of polysaccharide synthesis in liver cells, but our experiments suggest that the polysaccharides will be synthesized in supernatant, as neither mitochondrial nor microsomal fraction apparently contains any abnormal polysaccharides.

In any case, the results obtained in the present experiments show clearly that an abnormal metabolism of polysaccharides perhaps takes place in the liver of the cancer bearing individuals, and the polysaccharides deviated qualitatively from normal ones can be produced in the liver, suggesting that the metabolism of liver is severely affected by the metabolism of tumor cells.

#### SUMMARY

For the purpose to reveal whether or not the liver and the cell organellae are responsible for the abnormal metabolism of polysaccharides found in cancer bearing individuals, the author analyzed the liver and ascites with tumor cells of AH 130 hepatoma bearing rats biochemically with some histochemical observations. A quantitative increase in polysaccharides accompanied by the production of unusual polysaccharides is found in the supernatant of liver from cancer bearing rats, but not from mitochondrial or microsomal fractions. Tumor cells themselves and ascites fluid do not contain the abnormal polysaccharides found in the liver supernatant.

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