

THE CLINICAL BIOCHEMICAL INVESTIGATION
ON THE MALIGNANT BONE TUMOR
STUDIES ON THE VARIATION OF SERUM SIALIC ACID
AND HEXOSAMINE CONTENTS IN THE PATIENTS
WITH MALIGNANT BONE TUMOR
PRELIMINARY REPORT

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ABSTRACT

Serum sialic acid and total hexosamine values were remarkably elevated in the patients with malignant bone tumor and/or metastasis, in general, but in the great number of the patients with benign bone tumor both these levels remained within the normal range. Whereas, there were some cases with malignant bone tumor showing normal values in both serum sialic acid and total hexosamine levels, but there were no patients with benign bone tumor with values over 140 mg/dl in sialic acid and 180 mg/dl in total hexosamine.

Therefore, clinically, serum sialic acid and total hexosamine levels seemed to have some relation to the site and the pathological type of tumor. Serum sialic acid and total hexosamine levels had a tendency to decrease according to the effect of the treatment. However, the levels increased again after the recurrence and/or metastasis. Therefore, it may be considered valuable to estimate the serum sialic acid and total hexosamine levels to follow the response to therapy and to determine the prognosis. The positive correlation coefficient was mostly noted between the values of serum sialic acid and total hexosamine.

In the cases showing high glucosamine/galactosamine ratio, it was mostly caused by the change of glucosamine contents but hardly by the change of galactosamine contents.

INTRODUCTION

It has been well-known at the same time as the discovery of glycoprotein that serum glycoprotein levels manifested significant elevation in patients with malignant neoplasm. Numerous investigations have been carried out concerning serum glycoprotein levels in malignant tumor¹⁾²⁾, while the origin of serum glycoprotein has not yet been definitely explained. There are several concepts concerning the site of glycoprotein formation; it may be neoplasm itself, adjacent connective tissue of tumor, reaction to an unknown factor produced by

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malignant focus, or homeostasis of the human body. Among the investigators who explained the high serum glycoprotein levels released into the blood stream from neoplastic tissue, some have asserted that the increase of serum glycoprotein is a result of the proliferation of the malignant tumor tissue, or is also a result of the breakdown processes of malignant tumor.

Both could be accepted as factors in bringing about elevated serum glycoprotein levels, but they can not completely explain the mechanisms of these alterations and, even less, their significance⁽³⁾⁽⁴⁾⁽⁵⁾. Most of the experimental and clinical investigations have dealt with the changes of serum glycoproteins in the malignant tumor of soft parts; however, little attention has been paid to the changes of serum glycoproteins in malignant bone tumor⁽⁶⁾⁽⁷⁾. There has been a number of clinical reports in which alterations of serum glycoprotein levels in carcinoma of the gastrointestinal organs has been studied.

The variation may be influenced, primarily, not only by cancer itself, but also, secondarily, by some factors caused by gastrointestinal obstructions. Therefore, it is quite difficult to indicate exactly whether the serum glycoprotein values is being affected by cancer of soft tissues itself. However, it may be possible to show the exact alteration on serum glycoprotein contents which are affected by the tumor itself in the patient with malignant bone tumor before metastasis takes place in other organs. This investigation was undertaken to examine the changes of serum sialic acid and total hexosamine, monosaccharide constituents in glycoprotein, and moreover, ratio of glucosamine to galactosamine in patients with malignant bone tumor before and after treatment.

MATERIALS AND METHODS

Materials

Sera of 81 normal healthy subjects, aged 1 to 73 consisting of 46 males and 35 females, were estimated, and these estimated values were classified according to the age and sex (Table 1 and 2).

The number of 54 cases with bone tumor were assayed of these monosaccharide constituents in glycoprotein; there were 18 cases of sarcoma with bone lesion including osteogenic sarcoma, fibrosarcoma, malignant melanosarcoma, and rhabdomyosarcoma; 6 cases of giant cell tumor, 3 cases of bone cyst, 15 cases of cartilaginous exostosis, and 10 cases of metastatic carcinoma of the bones. As control, 24 cases of carcinoma of stomach, lung, mammary gland, and uterus were examined.

Blood samples were collected by venepuncture in the early morning from the patients with malignant neoplasm at intervals of one week before and after treatment. These sera were separated and kept frozen until they were analyzed. The changes of serum sialic acid and hexosamine levels, and the glucosamine/galactosamine ratio were observed during the course of the diseases.

TABLE 1. Effect of Age and Sex on Total Serum Hexosamine, Glucosamine/Galactosamine Ratio and Serum Sialic Acid Levels in 81 Normal Subjects, Aged 1 to 73

		1~20 Years	21~50 Years	51~73 Years
Number of analysis	Male	21	17	8
	Female	13	9	13
Hexosamine (mg/dl)	Male	110.6±6.2*	104.0±8.2*	113.6±8.3*
	Female	103.0±9.6	110.5±4.4	113.5±8.0
Gluco/Galacto ratio	Male	2.41±0.58	2.85±0.62	2.82±0.66
	Female	2.69±0.69	3.32±0.63	3.33±0.70
Sialic acid (mg/dl)	Male	58.5±8.0	61.7±11.2	62.8±6.7
	Female	55.9±8.4	62.7± 9.1	67.5±5.1

* Standard deviation of the mean.

TABLE 2. Results of Total Serum Hexosamine, Glucosamine/Galactosamine Ratio and Serum Sialic Acid Estimations in 81 Normal Subjects Classified by Sex

	Total	Male	Female
Hexosamine (mg/dl)	108.5±8.8*	108.6±8.5*	108.4±9.7*
Glucosamine/Galactosamine ratio	2.80±0.66	2.65±0.60	3.01±0.69
Sialic acid (mg/dl)	61.1±8.5	60.4±9.1	61.9±8.7

* Standard deviation of the mean.

Methods

As the procedure for assay of the total serum hexosamine, a modified Elson-Morgan reaction described by Boas (1953)⁹⁾, was used. That is, 0.5 ml of 6N-HCl was added to 1 ml serum, and the sample was hydrolyzed in boiling water bath for fifteen hours. After cooling, it was filtered into a column of Dowex 50-X 8, and the column was washed with 2N-HCl. Then, the elute was neutralized with 4N-NaOH.

The neutralized elution was used for an assay of total hexosamine, glucosamine and galactosamine.

Estimations of glucosamine and galactosamine were carried out with the method reported by Good *et al.* (1964)⁹⁾. The estimated values of glucosamine and galactosamine were simply shown as the ratio of glucosamine to galactosamine in this paper. The determination of serum sialic acid was made with the thiobarbituric acid assay by Warren (1959)¹⁰⁾¹¹⁾.

RESULTS

The normal serum values of sialic acid, total hexosamine and glucosamine/galactosamine ratio were indicated respectively with range of 47.5 to 72.6

mg/dl, 93 to 122 mg/dl and 1.83 to 4.03. There was no significant difference between the values of males and females (Tables 1, 2). The levels of serum sialic acid, hexosamine and glucosamine/galactosamine ratio in the patients with malignant bone lesion varied; among patients bearing sarcoma with bone lesion, some showed high contents of these components, and the others within a normal range. Therefore, clinically, the variation of these constituents may have a relation to the site, size, and pathological type of malignant neoplasm.

On examining the changes before and after treatment in various malignant bone focus, values of serum sialic acid and total hexosamine were found to be almost parallel. Before treatment, the higher values of serum sialic acid and hexosamine and glucosamine/galactosamine ratio were observed in the patients with cancer metastasis of the bone, osteogenic sarcoma, and malignant melanoma. On the contrary, the lower values of serum sialic acid and hexosamine and glucosamine/galactosamine ratio were observed in most patients with fibrosarcoma and alveolar soft part sarcoma, but a few was within normal limit. Minor change or normal range was obtained in the cases with benign bone tumor such as giant cell tumor, bone cyst, and cartilaginous exostosis before and after treatment. There were also many variations on the assay of the glucosamine/galactosamine ratio in malignant bone tumor, but the ratio reached high levels generally. The levels were variable during treatment of bone tumor (Figs. 1, 2, and 3).

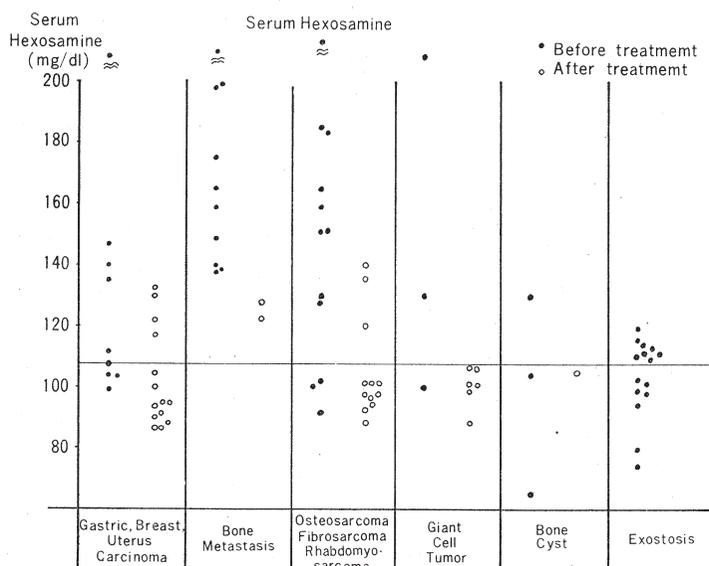


FIG. 1. Results of total serum hexosamine estimations in patients with bone tumor and/or other part tumor before and after treatment.

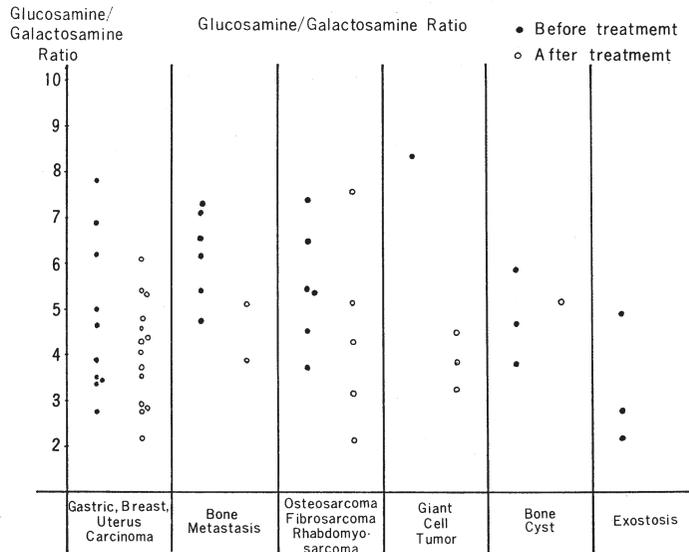


FIG. 2. Results of glucosamine/galactosamine ratio estimations in patients with bone tumor and/or other part tumor.

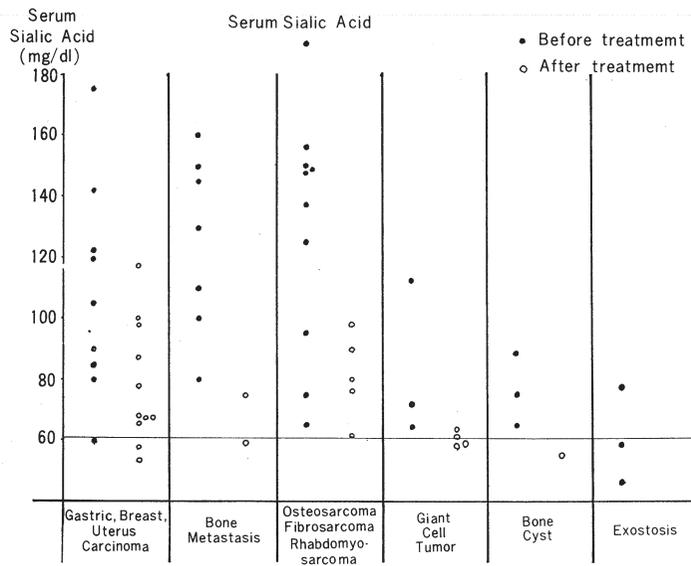


FIG. 3. Results of serum sialic acid estimations in patients with bone tumor and/or other part tumor before and after treatment.

Following is five of the cases:

(Case 1) H.M. male aged 27.

Osteogenic Sarcoma at the Lower Portion of the Right Femur.

Six months after surgical amputation, he visited our clinic complaining about swelling and pain in the stump. At that time, osteolytic findings and spicular formation at the stump and lung metastasis were found on the roentgenograms (Photo. 1).

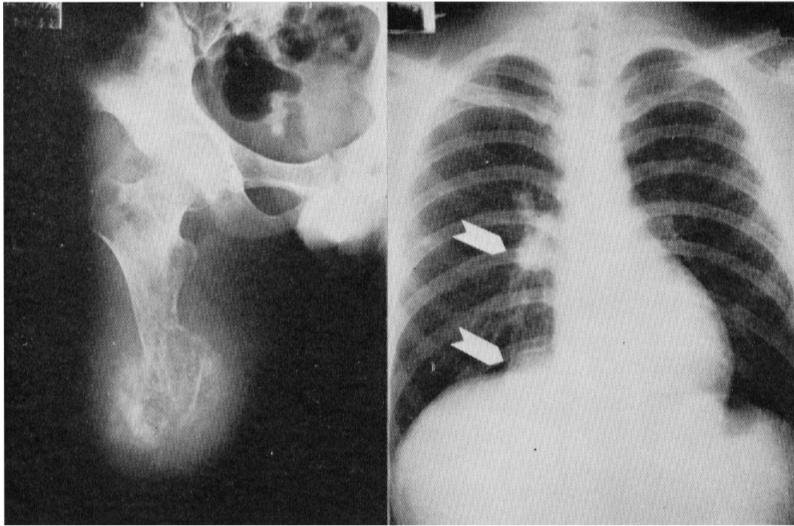


PHOTO. 1. Case 1, H.M. Male, Aged 27, Osteogenic sarcoma.

Osteogenic findings and spicular formation at the stump and lung metastasis are well observed on roentgenogram six months after surgical amputation.

During ^{60}Co -irradiation on the site with the total doses of 4,000 R and lung with the total doses of 2,000 R, the values of serum sialic acid and hexosamine temporarily increased, but later the values gradually decreased in relation to the clinical improvement. The glucosamine/galactosamine ratio also decreased (Fig. 4).

(Case 2) Y.T. female aged 52.

Fibrosarcoma at the Distal Part of the Right Femur (Fig. 5, Photo. 2).

When she visited our clinic for the first time, the distal part of the right femur had swollen considerably, and lung metastasis had already developed. Nevertheless, she had only slight pain in the distal part of the right thigh in spite of the large bone destruction on the roentgenogram. The patient was diagnosed to have fibrosarcoma at the distal portion of the right femur.

The femur and lung metastasis were exposed to local irradiation of ^{60}Co with the total doses of 3,000 R, 200 R daily. Clinical response had not been

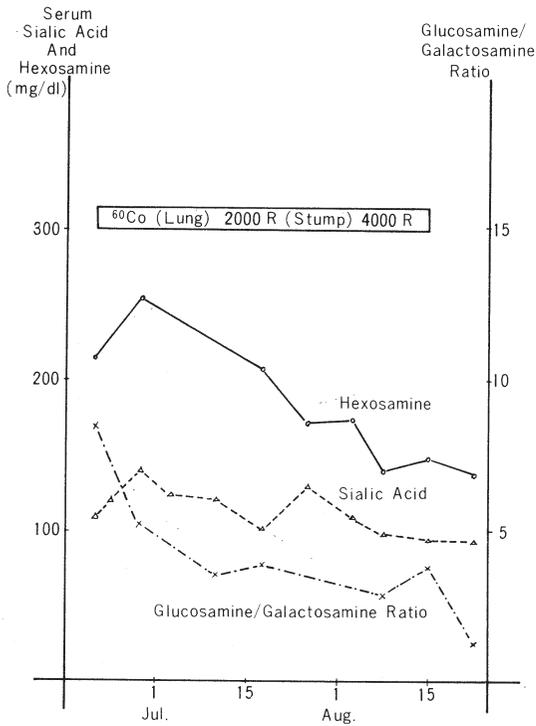


FIG. 4. Case 1, H.M. Male, Aged 27, Osteogenic sarcoma.

During ^{60}Co -radiation, the values of serum sialic acid and total serum hexosamine temporally increased, but soon after, gradually decreased with a parallel step to clinical improvement.

The glucosamine/galactosamine ratio was also on the decrease.



PHOTO. 2. Case 2, Y.T. Female, Aged 52, Fibrosarcoma.

The roentgen findings show osteolytic bone destruction at the lower portion of the right femur and lung metastasis.

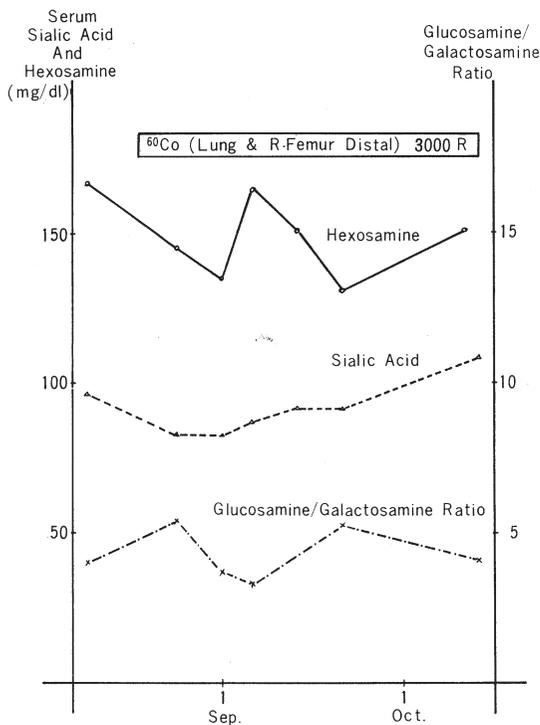


FIG. 5. Case 2, Y.T. Female, Aged 52, Fibrosarcoma.

A favourable clinical response had not been obtained together with high values of serum sialic acid and total serum hexosamine, and moderate ratio of glucosamine to galactosamine through ^{60}Co -radiation.

observed together with high levels of serum sialic acid and total hexosamine, and moderate glucosamine/galactosamine ratio.

(Case 3) T.S. male aged 34.

Alveolar Soft Part Sarcoma (Fig. 6, Photo. 3).

The focus at the left forearm, which had alveolar soft part sarcoma, was resected together with the distal part of ulna on May 8, 1964, and then amputation was done at the upper portion of the left upper arm on July 31, 1964. An osteolytic lesion was roentgenologically found at the left forearm. After operation, he had no complaint, but he began to complain of chest pain beginning in July, 1967. Remarkable lung metastasis were seen on the roentgenogram. ^{60}Co -irradiation with the total doses of 4,000 R had been given to the lung metastasis from August, 1967 and the cyclophosphamide (Endoxan, Shionogi Ph. Co. Ltd.) with the total doses of 2,000 mg were administered consecutively for twenty days starting with September, 1967. The values of serum sialic acid, total hexosamine, and glucosamine/galactosamine ratio remained within the normal range before and after treatment. Chest pain subsided after the treatments with constant values of serum sialic acid, hexosamine and glucosamine/galactosamine ratio.

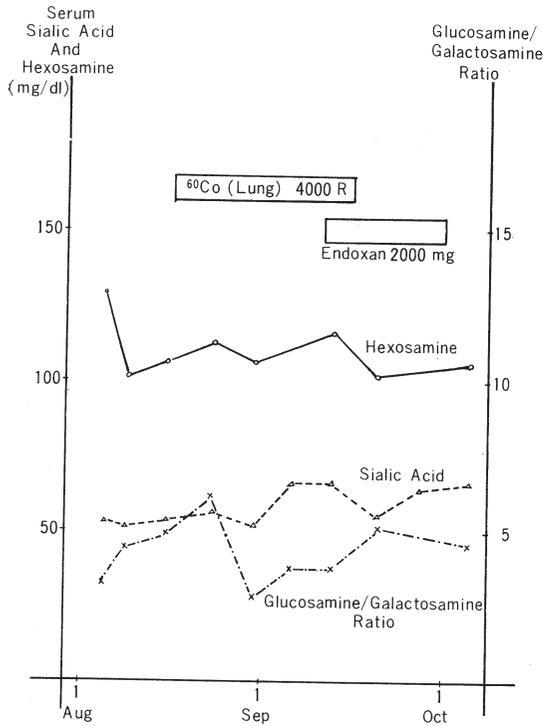


FIG. 6. Case 3, T.S. Male, Aged 34, Alveolar soft part sarcoma.

Treatments resulted in improvement of chest pain with constant values of serum sialic acid, total serum hexosamine and glucosamine/galactosamine ratio.

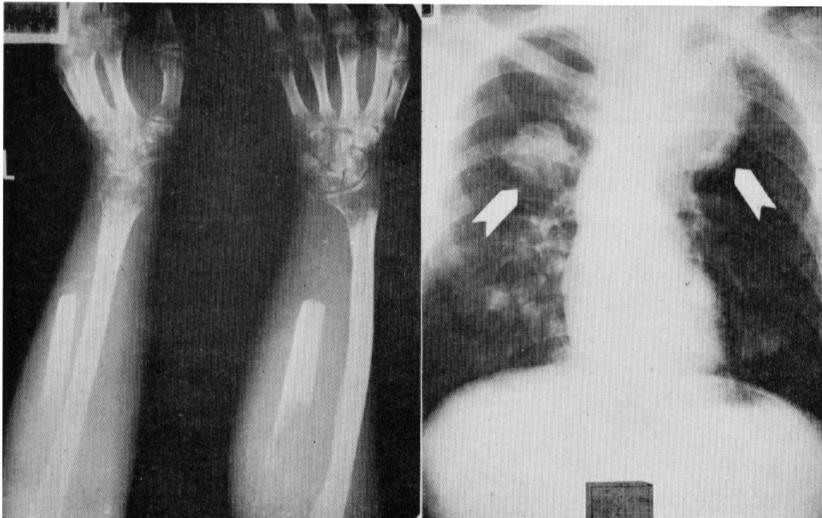


PHOTO. 3. Case 3, T.S. Male, Aged 34, Alveolar soft part sarcoma.

The roentgenogram show the relapse of the malignant focus at the left forearm two months after the first surgical operation (left) and the marked lung metastasis three years after the first surgery (right).

(Case 4) S.S. male aged 42.

Fibrosarcoma at the Upper Part of Right Fibula (Fig. 7, Photo. 4).

The patient had pain in the dorsal part of the right foot in March, 1967 which gradually increased, and the proximal part of the fibula expanded. The right femur was amputated at the level of the middle portion in August, 1967. The histological finding revealed fibrosarcoma. Serum hexosamine level showed

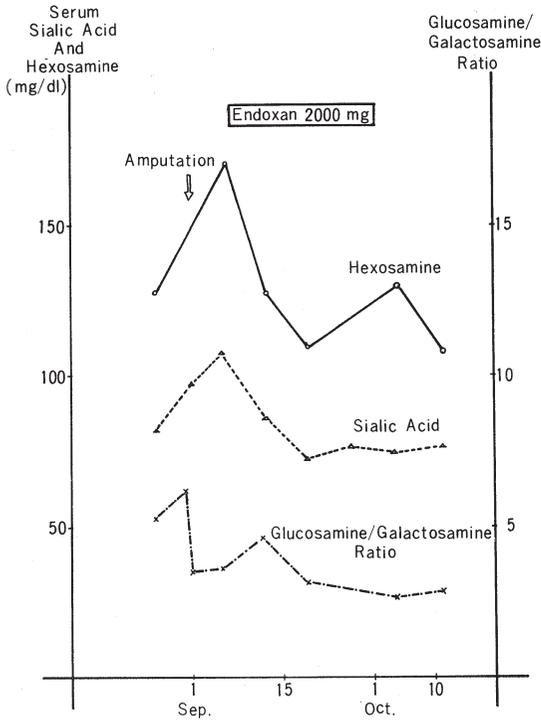


FIG. 7. Case 4, S.S. Male, Aged 42, Fibrosarcoma.

Serum sialic acid, total serum hexosamine and glucosamine/galactosamine ratio returned step by step to normal range after surgical operation, but these values were increasing with the appearance of metastasis at the 5th cervical spine.

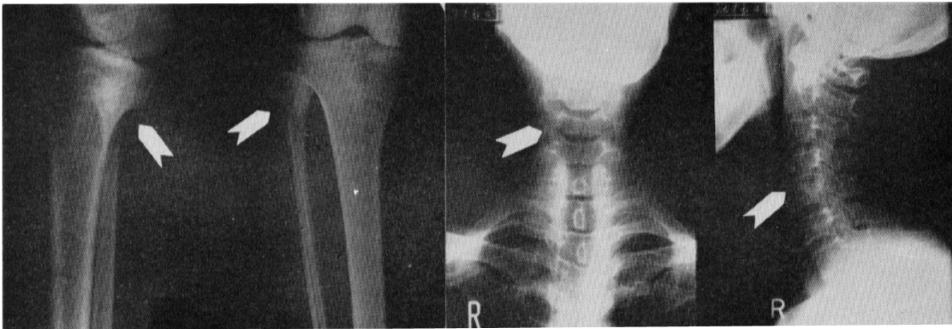


PHOTO. 4. Case 4, S.S. Male, Aged 42, Fibrosarcoma.

The X-ray (left) is at the first sitting and nearly one month after operation, the metastasis at the 5th cervical spine (right) was roentgenologically found.

170 mg/dl on the 5th day after the surgery, but step by step returned to normal range within a week, and the serum sialic acid level and glucosamine/galactosamine ratio also showed a similar tendency. Then, the patient complained of neck pain and metastasis at the 5th cervical spine appeared nearly one month later. Values of serum sialic acid, total hexosamine levels, and glucosamine/galactosamine ratio increased with the appearance of clinical symptoms.

(Case 5) T.E. male aged 59 (Fig. 8, Photo. 5).

Metastasis of Prostatic Cancer in the Pelvic Bone.

Since the spring of 1967, the patient had suffered from the left coxalgia and could not walk due to increasing pain. Sclerotic bone lesion was roentgenologically found at the left pelvic bone and movement of his left hip joint was restricted because of pain. He was admitted to our clinic on September to receive a course of intravenous injections of Honvan (stilbesterol-diphosphate-tetrasodium; Kyorin Phar. Co. Ltd.) in a dose of 200 mg every other day. The pain decreased after four injections, and he was able to walk. The serum hexosamine value before injection was high, namely, 190 mg/dl, and gradually decreased to 170 mg/dl on the 5th day, 155 mg/dl on the 12th day, and 120 mg/dl on the 18th day after the treatment. Serum sialic acid level showed 135 mg/dl before injection and decreased after the treatment; 120 mg/dl on

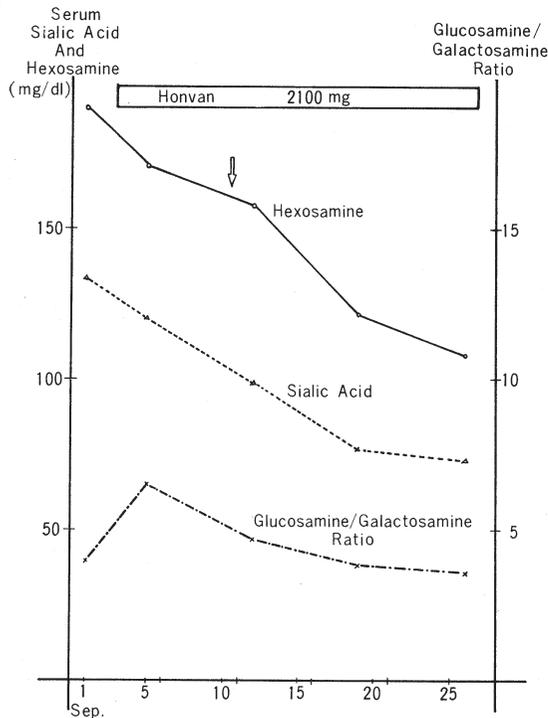


FIG. 8. Case 5, T.E. Male, Aged 59, Metastasis of prostatic cancer.

As clinical findings were improved by treatments, the values of total serum hexosamine tended consequently to decrease as parallel as the levels of serum sialic acid and glucosamine/galactosamine ratio.

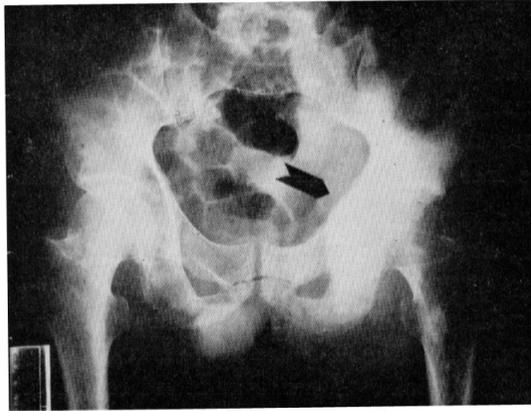


PHOTO. 5. Case 5, T.E. Male, Aged 59, Metastasis of prostatic cancer.

The roentgenogram shows sclerotic bone lesion in the left pelvic bone.

the 5th day, 98 mg/dl on the 12th day, and 78 mg/dl on the 18th day. There was a decreasing tendency in glucosamine/galactosamine ratio as well.

Summing up these five cases: With improvement of the clinical findings from the treatments, the values of serum hexosamine and serum sialic acid levels had a tendency to decrease in parallel in cases 1 and 5. On the other hand, when the treatment was ineffective in cases 2 and 3, the serum sialic acid and total hexosamine showed continuously high levels. Both cases 2 and 3, the serum sialic acid and total hexosamine showed continuously high levels. Both cases 2 and 3 suffered from fibrosarcoma. In case 3, after surgical amputation, the values of serum sialic acid and hexosamine decreased slightly but did not return to normal range. It may be due to the metastasis development on the 5th cervical spine. Because large lung metastasis existed, these values did not decrease in case 2.

As shown in the results, the alteration of serum sialic acid and total hexosamine levels showed various degree in patients with malignant bone tumor under different therapy.

DISCUSSION

Scrutinizing the results presented in this paper, it should be admitted that estimation of serum sialic acid and total hexosamine is useful as an aid in establishing the diagnosis of malignant bone tumor to some extent. Because in every group of patients with malignant bone focus, we found only a few cases showing the values within normal ranges. But in most cases of malignant bone tumor, the patients had high values reaching over 180 mg/dl in total serum hexosamine and over 140 mg/dl in serum sialic acid. On the other

hand, there were no patients with these high levels in cases of benign bone tumor, except one patient with giant cell tumor of the 3rd grade which had a high level of total serum hexosamine. Therefore, patients with these high values over 180 mg/dl in total serum hexosamine and over 140 mg/dl in serum sialic acid, give suspicion of malignant tumor—if there are no complications of inflammation, collagen disease, or other diseases. But these indications do not necessarily mean there is not the malignant focus in the case even though these values are within normal ranges.

Weiden (1958)¹²⁾ mentioned that very high values of serum hexosamine were obtained in four of five patients with Hodgkin's disease before treatment, namely, 210, 228, 229, and 260 mg/dl, and the high level of serum hexosamine in Hodgkin's disease could be used as an aid in the diagnosis of this disease. Spiero, *et al.* (1966)¹³⁾ estimated the values of serum hexosamine in malignant lymphoma, leukemia, carcinoma, and melanoma and reported that the estimation of the serum hexosamine level could seemingly have only limited values as an aid to diagnosis for these diseases. It seems impossible to use the determination of serum glycoprotein contents as an aid to diagnosis for the specific malignant neoplasm, because high values of serum glycoprotein were obtained also in patients with inflammation, collagen disease, and other diseases. Nevertheless, in patients with suspected malignant bone tumor by X-ray examination, clinical pictures, and laboratory tests, it may be considered valuable in observing the response to therapy, in prognosis, and also for diagnosis of malignancy such as osteogenic sarcoma or fibrosarcoma, and to evaluate serum glycoprotein levels and variations of serum glycoprotein during treatment.

Carcinoma originates from epithelial cells, while sarcoma consists of mesenchymal tissue. The metabolism of carcinoma cells seems to differ from that of sarcoma cells.

Werner (1953)¹⁴⁾ represented in his "Studies on glycoprotein" that all mucous secretions contain also a sialoprotein, *i.e.* a glycoprotein containing sialic acid as characteristic sugar component. So his investigation is worthwhile to study monosaccharide constituents in glycoprotein.

Danishesky *et al.* (1966)¹⁵⁾ studied the mucopolysaccharide content of 4 types of animal tumor: Flexner-Jobling carcinoma, Walker carcinosarcoma 256, rat fibrosarcoma and sarcoma 180. Then, they reported that largest amounts of mucopolysaccharides were found in the induced fibrosarcoma and in the Flexner-Jobling carcinoma, but sarcoma 180 and Walker carcinosarcoma 256 contained minute amounts.

Relationship between neoplasm and its interstitial tissue has been recently noted, and also the relationship between the amount of mucopolysaccharide and fibroblast was described by Danishesky *et al.*¹⁵⁾ Relationship between the histological differences and the variations of serum and tissue glycoprotein levels should be taken into account for further study in the malignant tumor.

The problems concerning osteolytic focus and osteoplastic focus are interesting enough.

Keyser (1954)¹⁶⁾ said that a favourable clinical response was generally associated with a fall in the blood serum polysaccharide (total mucoprotein) towards normal levels in patients with malignant tumor treated with di(2-chloroethyl) methylamine hydroride or by radium or X-irradiation. However, without clinical improvement these values of serum sialic acid and total hexosamine to normal range could not be held. These results would indicate the estimations of serum sialic acid and total hexosamine levels as a good index observing the response to therapy and in prognosis. In a few cases, the levels of serum sialic acid and total hexosamine increased temporarily as shown in Case 1 after ⁶⁰Co-irradiation. If the evaluation of these constituents were frequently made after ⁶⁰Co-irradiation, the temporary increase of these values would be obtained in many cases. The elevations of these values soon after X-irradiation may be caused by the stress of radiation or reactions of malignant focus to irradiation (Boas *et al.*¹⁷⁾ 1953). The similar high levels after X-irradiation were observed by Keyser¹⁶⁾ in his clinical investigations and also by Nakagawa *et al.* (1967)¹⁸⁾ in the experimental bone tumor induced by the transplantation of Walker carcinosarcoma 256.

The glucosamine/galactosamine ratio in total serum hexosamine showed generally high levels in patients with malignant bone tumor, but these estimated values were of wide range. In the experimental study, Nakagawa *et al.*¹⁸⁾ observed that the ratio was remarkably elevated in sera of rats with induced bone neoplasm compared with controls. Also it was reported by Kanishefsky *et al.*¹⁵⁾ that the fibrosarcoma and the Flexner-Jobling carcinoma contain appreciable amounts of hyaluronic acid and chondroitin-sulfate. Variations of glucosamine and galactosamine levels in connection with clinical symptom may be the problem. In the present results, high levels of serum sialic acid and total hexosamine were commonly found in patients with metastasis in bony tissue. Therefore, present authors may infer that there is certainly a relationship between these values of serum sialic acid and total hexosamine levels and the site, size, and prognosis of malignant neoplasm.

Nakagawa *et al.*¹⁸⁾ have suggested that the positive correlation coefficient exists between the wet weight of subcutaneous tumor masses induced by the transplantation of Walker carcinosarcoma 256, and the levels of serum sialic acid and total hexosamine in the experimental study. But clinically, the elevated levels of total serum hexosamine were not always found in patients with metastasis of malignant tumor (Spiero *et al.*¹³⁾ 1966). In this analytical data, it was found that variations of serum sialic acid and total hexosamine were generally parallel.

CONCLUSION

1) Serum sialic acid and total hexosamine values, and glucosamine/galactosamine ratio were remarkably elevated in patients with malignant bone tumor and/or bone metastasis.

2) Serum sialic acid and total hexosamine contents, and glucosamine/galactosamine ratio remained within normal range in some cases with malignant bone tumor and/or bone metastasis, but there were no cases showing the value over 140 mg/dl in sialic acid and 180 mg/dl in hexosamine in patients with benign bone tumor.

3) Generally, serum sialic acid and total hexosamine levels, and glucosamine/galactosamine ratio had a tendency to decrease according to the effect of treatment and the improvement of clinical symptoms.

4) It may be considered valuable to determine serum sialic acid and total hexosamine levels in patients with bone tumor in observing the response to therapy in prognosis and for the early diagnosis of recurrence and/or metastasis.

5) In the case showing high glucosamine/galactosamine ratio, it was mostly caused by the change of glucosamine content but hardly by the change of the galactosamine level.

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