Short Communication

GLYCOSAMINOGLYCAN-SYNTHETIC ACTIVITY OF NEOPLASTIC AND NON-NEOPLASTIC ADIPOSE TISSUES

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In well-differentiated liposarcoma there are commonly observed mucinous areas that have merged with a vascular tissue, mainly composed of small and large lipocytes of varying degrees of maturity. It was demonstrated by biochemical and histochemical investigations that the liposarcoma tissue contained glycosaminoglycans (GAG) consisting mainly of hyaluronic acid (Meyer, 1955; Winslow & Enzinger, 1960; Kaneko, 1974; Kindblom & Angervall, 1975; Filipe & Mackenzie, 1976) but a precise analysis of the synthetic activity of GAG in adipose tissue has not been performed. In the present study, we observed the GAG-synthetic activity of neoplastic and non-neoplastic adipose tissues by examining the incorporation of 3H-glucosamine or 35SO4 into tissue GAG.

Each tissue used for analyses is shown in the Table.

Immediately after surgical excision, each tissue was cut into thin slices (1 mm thick) and the slices were incubated in the following medium: 10% dialysed calf serum (Research Institute for Microbial Diseases, Osaka University) in Eagle’s minimal essential medium (F-12, GIBCO, Grand Island, N.Y., U.S.A.) containing 10 μCi of 35SO4/ml (sp. act. 0·33 Ci/mmol) or 10 μCi of 3H-glucosamine (sp. act. 21 Ci/mmol). After 1h incubation at 37°C, the tissue slices were washed with 80% ethanol, embedded in paraffin and sectioned. The sections were stained with Alcian blue–haematoxylin and eosin, covered with photographic emulsion (SAKURA NR-H2, Konishiroku Photo Industries Co. Ltd, Tokyo) and an autoradiograph was made for the localization of labelled materials in the tissues.

Procedures to identify 35S- or 3H-labelled materials in the tissue segments after incubation (see above) were essentially the same as described in our previous papers (Takeuchi et al., 1978; Sobue et al., 1980). The labelled tissue segments were washed with 80% aqueous ethanol several times to remove free isotopes, and dried with acetone. After being weighed, the resulting dry powder was suspended in 0·3m NaOH and kept at 4°C overnight. It was then neutralized with 1m HCl, adjusted to pH 8·0 with 1m Tris-HCl buffer, and digested with pronase. Undigested materials in each tube were discarded by centrifugation at 3000 rev/min for 15 min. Glycosaminoglycans were precipitated from the supernatant by adding 3 volumes of 95% ethanol containing 1% potassium acetate. Ethanol precipitation was repeated 3 times, and the precipitate was washed with 80% ethanol, and acetone dried. The materials obtained were dissolved in 0·1 ml of water per mg dry weight of the tissue. An aliquot was taken to determine the uronic acid concentration

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TABLE.—Radioactivity of $^{3}H$-hyaluronic acid, $^{3}H$-sulphated glycosaminoglycans, $^{35}S$-dermatan sulphate and $^{35}S$-chondroitin sulphate in each tissue (ct/min/mg of defatted tissue)

| Case | Age | Sex | Histological diagnosis                        | $^{3}H$-hyaluronic acid | $^{3}H$-sulphated GAG | $^{35}S$-dermatan sulphate | $^{35}S$-chondroitin sulphate | U.A.* |
|------|-----|-----|-----------------------------------------------|-------------------------|-----------------------|---------------------------|-------------------------------|-------|
| 1a   | 71  | M   | Liposarcoma with myxoid area†                 | 4872                    | 663                   | 330                       | 62                           | 277–355 |
| 1b   | 71  | M   | Liposarcoma with myxoid area‡                 | 2231                    | 248                   | 529                       | 302                          | 146–148 |
|      |     |     | Subcutaneous adipose tissue (abdominal wall)  | 40                      | 50                    | 113                       | 94                           | 7.3–7.4 |
| 2    | 18  | F   | Lipoma (size of an egg) (subcutane, shoulder) | 172                     | 211                   | 256                       | 63                           | 23–25.6|
| 3    | 71  | M   | Lipoma (size of a fist) (subcutane, back)     | 42                      | 38                    | 469                       | 239                          | 10.9–11.0|
| 4    | 43  | M   | Adipose tissue (omentum)                      | 20                      | 43                    | 289                       | 64                           | 11.2–11.8|
|      |     |     | Subcutaneous adipose tissue (abdominal wall)  | 14                      | 16                    | 133                       | 49                           | 8.8–11.1|
| 5    | 58  | M   | Adipose tissue (omentum)                      | 20                      | 31                    | 110                       | 125                          | 10.4–11.0|
|      |     |     | Subcutaneous adipose tissue                   | 39                      | 39                    | 11                        | 19                           | 6.3–6.8 |

* The range of uronic acid content (nmol/mg of defatted tissue) in 3 pieces of each tissue.
† Tumour tissue (4.3 kg) from the retroperitoneal space, and occupying the abdominal cavity.
‡ Recurrent tumour tissue (5.8 kg) extirpated about 8 months after the excisions of Case 1a.

After defatted tissue was treated with 0.3 N NaOH and digested with pronase, an aliquot of the resulting homogenate was subjected to a paper chromatography with butanol/acetic acid/0.5 M ammonia (2:3:1 v/v) in which GAGs had little mobility, as described in our previous paper (Sobue et al., 1980). About 80–90% of the radioactivity remaining at the origin of the paper chromatogram could be detected in the GAG fraction.

by the procedure of Bitter & Muir (1962). Each solution containing 10 nmol of uronic acid was spotted on a cellulose-acetate membrane and electrophoresed under the condition described in our previous paper (Sobue et al., 1978). GAG components (hyaluronic acid, chondroitin sulphate, dermatan sulphate and heparan sulphate) were separated from each other by this procedure. The strips were stained with 0.2% Alcian blue in 0.1% acetic acid. Radioactivity incorporated into the individual component of glycosaminoglycans, which were fixed and stained on the electrophoresis strip with Alcian blue, was measured by cutting the strips and placing them in vials with a scintillation solution as described in our previous paper (Sobue et al., 1978). Further identification of GAG components was performed enzymatically. Each solution containing 50 nmol of uronic acid was digested with chondroitinase ABC, chondroitinase AC and hyaluronate lyase, respectively, under the optimal conditions described in the indicated literature (Yamagata et al., 1968; Hiyama & Okada, 1975; Ohya & Kaneko, 1970). The enzyme-treated and untreated materials were submitted to cellulose-acetate membrane electrophoresis (see above).

Malignant neoplastic tissue used for analysis was the well-differentiated liposarcoma, composed of the mature fat cells with wide areas of myxoid tissue. In the myxomatous matrix, stained profusely

![Fig. 1.—Microscopic section of liposarcoma tissue (Case 1) showing a myxomatous pattern (Alcian blue and H & E. ×240).](image-url)
with Alcian blue, the stellate and fusiform cells were arranged into a loose network, as shown in Fig. 1. In some areas, a few rounded lipoblasts and bizarre lipoblasts with an abnormal nucleus were seen. In an autoradiograph, diffuse $^{35}$S-radioactivity was observed in the tumour cells of the myxomatous areas, as shown in Fig. 2, and the amount of $^{35}$SO$_4$ incorporated in the vascular cells was relatively large. Diffuse and low $^3$H-radioactivity was also seen in the tumour cells. In the case of lipoma consisting of mature lipocytes, the level of $^3$H-radioactivity observed in the tumour cells was not so high, and the amount of $^{35}$S labelling was significant in the vascular cells (Fig. 3). In the non-neoplastic adipose tissues, $^{35}$S labelling was relatively low.

The incorporation of $^3$H label into hyaluronic acid (ct/min/mg of defatted tissue) and that of $^3$H and $^{35}$S label into sulphated GAGs in each tissue is shown in the Table. Hyaluronic acid synthesis by the sarcoma tissue was 50–200 times that of the lipoma or the non-neoplastic adipose tissue. The GAG content of the sarcoma tissue was 10–30 times that of lipomas and adipose tissues. However, differences in $^{35}$S-sulphated GAG synthesis between the sarcomatous tissue, the lipomas and the adipose tissues were not so significant, though the activity was higher in the neoplastic tissues. $^{35}$S-dermatan sulphate synthesis was in most cases considerably higher than $^{35}$S-chondroitin sulphate synthesis.

The results of the autoradiography suggested that the amount of sulphated GAGs synthesized by the adipose tissues may relate to the amount of the vascular components, which were diffusely distributed in these tissues, whereas most of the hyaluronic acid detected was synthesized by the lipoblasts. There are two theories concerning the histogenesis of adipose tissue: (1) Adipose cells are merely fibroblasts which have accumulated excess lipid. (2) Adipose tissue develops from lipoblasts which became separated from mesenchymal cell early in embryonic life. It is well known that sulphated-GAG synthesis is one of differentiation of mesenchymal cells (Lovell et al., 1966; Prodi & Romeo, 1967), and it was noted that hyaluronic acid is usually synthesized by fibroblastic cells only during the actively growing phase (Morris, 1960; Davidson, 1963). The present result, showing that hyaluronic acid production has a close relation to proliferation of lipo-
blasts, seems to indicate that the biological characteristic of lipoblasts and fibroblasts are similar in terms of GAG synthesis. Recently, in our laboratory, we observed GAG synthesis by subcutaneous mesenchymal tissues of SMA mice, by the procedure described in the present study. The interscapular brown fat tissue revealed a high level of GAG-synthesis. The amount of $^3$H label incorporated into the GAG of brown fat (ct/min/mg dry tissue) was 1.4–3.0-fold higher than that of the subcutaneous loose connective tissues.

It was reported that hyaluronate, a major component of extracellular matrices through which cells migrate during embryonic tissue development and in regeneration, is also concentrated in the environment through which neoplastic cells invade local host tissues (Toole et al., 1979). It was demonstrated in our previous studies that sulphated GAG, as well as hyaluronic acid, support the viability of tumour cells in vivo and in vitro (Takeuchi, 1966, 1972; Takeuchi et al., 1974). It is conceivable that in the tumour presented here the growth of tumour cells is facilitated by GAG produced by the tumour cells themselves.

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