It was reported that concentration of $^{67}$Ga was predominant in inflammatory tissues rather than in viable tumor tissue, and $^{67}$Ga was concentrated into lysosome in the liver. This study was undertaken to elucidate $^{67}$Ga binding substances in tumor tissue and in the liver lysosome.

1. Material and Methods

$^{67}$Ga-citrate was injected to the mouse implanted with Ehrlich tumor. The tumor tissue and liver were excised at 24 hours after administration of $^{67}$Ga-citrate. These tissues were rinsed in 0.01 M tris—0.15 M KCl buffer solution and then homogenized. Nuclear fraction was removed from the homogenate of tumor tissue by centrifugation. This homogenate from which nuclear fraction was removed, was incubated with pronase P in buffer solution (pH 8.0) at 37°C for 48 hours. On the other hand, subcellular fractionation of the liver was carried out according to the method of Hogeboom and Schneider. Mitochondrial fraction (lysosome was contained in this fraction) was also incubated with pronase P in buffer solution at 37°C for 48 hours.

After digestion, the reaction mixtures were gelfiltered on Sephadex G-100. Eluate samples were collected in an automatic fraction collector and assayed for $^{67}$Ga, uronic acid (by orcinol method) and protein (by Lowry’s method).

The eluates of the first and the second peaks (Fig. 1) obtained by Sephadex G-100 gel filtration were concentrated to a small volume and cellulose acetate electrophoresis of these fractions was carried out in veronal buffer solution (pH 8.0). Thereafter, cellulose acetate strips were dried at room temperature and placed on X-ray film. This film was developed after the exposure of several days. Cellulose acetate strips were then stained with alcian blue.

On the other hand, the eluates of the first peak and second peaks obtained by Sephadex...
G-100 gelfiltration were incubated with DNase and RNase in 0.1 M acetate—0.15 M NaCl buffer solution (pH 5.0) at 37°C for 3 hours. After incubation, the reaction mixtures were gelfiltered on Sephadex G-50. Eluate samples were collected in an automatic fraction collector and assayed for ⁶⁷Ga, uronic acid and protein.

2. Results and Discussion

Figure 1 illustrates the results of Sephadex G-100 gelfiltration in the case of tumor tissue. Three peaks of ⁶⁷Ga are shown in this figure. First peak, containing macromolecules bound to ⁶⁷Ga, is in void volume, and third peak is in the low molecular substances. Intermediate molecular substances bound to ⁶⁷Ga (second peak) was separated by Sephadex G-100 gelfiltration between first peak and third peak.

In the case of cellulose acetate electrophoresis of second peak of Sephadex G-100 gelfiltration, the band in autoradiogram of cellulose acetate strip was identical with the band stained with alcian blue (Fig. 2). But, in cellulose acetate electrophoresis of first peak of Sephadex G-100 gelfiltration, there were bands in autoradiogram of cellulose acetate strip, and these bands were identical with the bands stained with alcian blue.

On the other hand, digestion of elutes of first and second peaks with DNase and RNase did not liberate ⁶⁷Ga from macromolecules. Very similar results were also obtained in the case of lysosome of the liver.

From these experiments, it was concluded that there was a kind of acidic mucopolysaccharide, which had not uronic acid in its structure, bound to ⁶⁷Ga in second peak and there were some kinds of acidic mucopolysaccharides bound to ⁶⁷Ga in first peak on Sephadex G-100 gelfiltration, and that ⁶⁷Ga was not bound to protein and nucleic acid in eluates of first and second peak. As for liver lysosome, very similar results were also obtained.

From the above-mentioned facts, it was revealed that ⁶⁷Ga was bound to the acidic mucopolysaccharides in the tumor tissue and liver lysosome.

References

1) A. Ando, et al.: Radioisotopes, 26, 421 (1977)