Fibrin in Human Tumors
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SUMMARY

Sections of human tumors have been stained for fibrin deposits by the fluorescein label technic. Large clots of extravascular fibrin were demonstrated in most of the tumors tested. No such large clots were found in the several normal tissues investigated. However, there was some staining of certain areas of connective tissue in both normal and tumor tissues. The fibrin deposition observed in the spontaneous human tumors was very similar to that of the several transplanted rat tumors. This observation of the presence of fibrin does not give information about the rate of fibrin deposition.

Radiolabeled antifibrin antibodies when injected into tumor-bearing rats have been shown to localize in the tumor in the case of several transplanted neoplasms (3, 4, 8-11). This appears to take place by the interaction of the labeled antifibrin antibody with fibrinogen in the circulation and subsequent simultaneous deposition of antibody and fibrinogen in the transplanted tumor. Indeed we have shown by radiolabel that fibrinogen itself is fixed in the rat tumor, presumably as fibrin (4). Moreover, the presence of fibrin in extravascular areas of tumor has been demonstrated by fluorescein label technics (8). The possibility of fixing radioactivity in human tumors by use of radiolabeled antihuman fibrin antibodies would depend on the presence of fibrin in the neoplasm. Therefore, we have examined several human tumors for fibrin deposits by the fluorescein label technic and are reporting the results here.

MATERIALS AND METHODS

Antisera.—Rabbit antihuman fibrinogen serum was obtained from Dr. Y. Yagi. Rabbits had been given injections of fibrinogen (85-90 per cent clottable) obtained by the method of Biggs and MacFarlane (1). As control sera, rabbit antihuman globulin serum (6) and pooled normal rabbit serum were used.

Tumor tissues tested.—Human tumors were obtained from surgery or necropsy. All tissues were stored frozen immediately upon arrival and employed as required. The diagnoses listed are those from the pathology reports.

Fluorescent staining technic.—Coons' indirect staining technic was used (2). All sera as well as the fluorescein-labeled horse antirabbit globulin reagent were subjected to absorption 3 times with human liver sediments and once with rat liver sediment to remove nonspecific staining components as described previously (7).

To demonstrate that antifibrinogen serum contained antibodies to fibrin, a portion of the serum was treated with a slight excess of fibrinogen (Pentex fraction I) to precipitate antifibrin antibody. The supernate was treated subsequently with washed powdered fibrin. The absorbed antiserum was employed for staining and showed a marked drop in staining of washed fibrin clots. The unabsorbed serum gave an intense staining of the clot.

RESULTS AND DISCUSSION

Several tumors and several specimens of normal tissues were sectioned and stained. They were treated with both antifibrin serum and with control sera. In both tumor and normal tissues, staining in blood vessels was observed with antifibrin serum. This was apparently due to fibrin elements which were present in the vessels because the tissues had not been perfused. In addition, most tumors showed intense staining of fibrin aggregates throughout the tumor, particularly in the necrotic areas. Not all tumors showed the staining, however. There was no staining of any tumor cells

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592
All tissues were stained with fluorescein-labeled horse anti-rabbit globulin after prior treatment with rabbit antihuman fibrinogen serum.

**Fig. 1.** Lymphosarcoma II—the fibrin aggregates were dispersed throughout the section; tumor cells were unstained. The staining here was similar to that seen with lymphosarcoma I.

**Fig. 2.** Lymphosarcoma III—staining of fibrin areas occurred particularly in large and small vascular channels, and essentially no fibrin clots were seen outside of these vessels. The lymphosarcoma cells were unstained.

**Fig. 3.** Osteogenic sarcoma—large and small fibrin deposits were found throughout the section.

**Fig. 4.** Malignant melanoma—areas containing fibrin-like elements were stained. Large malignant melanoma cells in the background were unstained.

**Fig. 5.** Carcinoma of the breast—small aggregates of fibrin observed stained among the tumor cell nests. Some of these can be seen within vessels and along the connective tissue. The tumor cells which can be seen in the photograph were unstained.

**Fig. 6.** Choriocarcinoma of the testis—fibrin clot seen among the tumor cells; other areas of the section showed small clots in vessels and others staining stroma.

**Fig. 7.** Leiomyosarcoma—isolated clots of fibrin stained strongly among the unstained smooth muscle tumor cells. Most of the fibrin observed here was located within vascular channels.

**Fig. 8.** Rectal carcinoma—necrotic areas containing degenerating tumor cells were stained, while nests of healthy appearing tumor cells were not. Normal smooth muscle tissue present in the section was unstained.
themselves. There was no particular distribution pattern of fibrin that could be considered important; in certain areas of a tumor tissue section, aggregates and huge clots were present, whereas a few or none was seen in adjacent regions. Only a few sections of a small part of any one tumor were studied, and no attempt was made to survey completely a whole tumor. In the figures, areas containing fibrin-like elements are seen in the various tissues (Figs. 1–8).

Three lymphosarcomas were studied. Two gave positive fibrin staining in places other than within vessels, while the third was negative. Two melanomas were studied, and only one showed extravascular staining.

The following tumors were also studied, and extravascular fibrin deposits were found in all: an osteogenic sarcoma of the left femur, two Hodgkin’s disease lesions, a carcinoma of the stomach, three carcinomas of the breast, two Wilms’ tumors, two testicular tumors, a leiomyosarcoma of the uterus, and a rectal carcinoma.

In some tumors, namely a lipoma, a malignant melanoma, and a mesothelioma, appreciable extravascular fibrin deposits were not seen. This does not necessarily mean that there were none elsewhere in the tumor since only a small part of the whole tumor was sectioned.

Normal tissues (three testes, three kidneys, two spleens, one liver, and one smooth muscle) showed no marked extravascular fibrin deposits except for some connective tissue staining and perhaps in the spleen. Gitlin et al. (5) reported finding fibrinogen in the lymphatic and vascular channels, connective tissue, and interstitial spaces. In Gitlin’s work, the tissues were treated with alcohol and acetone prior to exposure to aqueous media, while in ours the sections were air-dried and treated directly. It may be that in Gitlin’s preparation the nonaqueous solvent treatment may have fixed the fibrinogen present, giving more areas of staining than were observed in our preparations. Tissue sections were treated with control sera, antihuman gamma globulin and pooled normal rabbit serum. The latter gave essentially no staining. Antihuman gamma globulin gave weak staining of stroma and fibrin clots in some tissues or a weak greenish fluorescence over the whole section. In general, however, staining was different from that observed with antifibrinogen serum.

Thus far several spontaneous human tumors have been found to contain extravascular fibrin deposits. In this they parallel transplanted rat tumors. In the case of transplanted rat tumors, the actual rate of fixation of fibrinogen and antifibrinogen antibody depends upon rapid growth of the tumor (4). Such rapid growth may not obtain in most spontaneous tumors. It remains to be seen whether fibrin is laid down rapidly enough in spontaneous tumors to permit appreciable localization of radioiodinated fibrinogen or antifibrin antibody.

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