

THE DEMONSTRATION OF A TUMOR GROWTH-IN-  
HIBITING FACTOR FROM NORMAL HUMAN  
CONNECTIVE TISSUE

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Murphy and his coworkers among others have shown that an inhibiting factor can be isolated from fowl sarcoma, capable of neutralizing the causative agent of this tumor (1). This factor also prevents the growth of transplantable mouse sarcomas, thus demonstrating that it is not a species-specific agent (2). In a more recent study these investigators have further extended their search for agents which have the power of inhibiting growth in transplantable mammalian carcinomas. They have found that extracts from normal animal embryo skin and placenta yield substances which exert definite retarding action on two transplantable carcinomas of mice, but have no effect on mouse sarcomas (3, 4). No definite inhibition was noted in fresh extracts, desiccation of the material being necessary to produce the optimum inhibitory effect.

As soon as the methods were available, an investigation was undertaken in our laboratories on sources of inhibitors in human tissues. We have studied extracts of fresh human placenta, of pectoral muscle, of rectus sheath, and of uterine fibroids. After some preliminary trials it seemed best to limit our work to one type of tissue extract and to defer experimentation on the other tissues to a later date. Since the extracts of human connective tissue (rectus sheath) have indicated more of promise than the others, we confined our attention to these.

*Material and Method*

In all experiments white rats approximately 4 months of age were used. These rats were bred from our own stock strain. This strain has been used extensively here in connection with the test tumor and is not resistant to it. The rat tumor,

No. 256, used has consistently shown over 90 per cent successful grafts in a long series of experiments carried out by ourselves and other investigators in The University of Rochester Medical School. It attains on the average a size of 3.0 x 1.5 cm. at the end of a 5 weeks period in control animals. Extracts of fresh human fascia were used in all of our experiments. The tissues were obtained either from recently deceased patients in the course of routine post-mortem

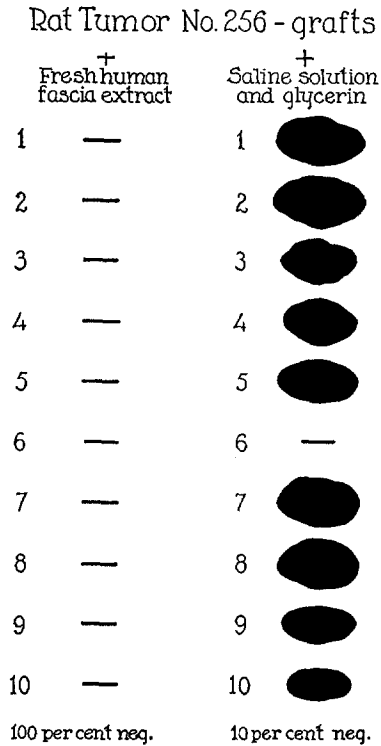


FIG. 1. Complete inhibition of the tumor growth was obtained in three such experiments. Silhouettes taken 5 weeks after grafting in all cases. The silhouettes are one-third the size of the growths.

examination or directly from the operating room. The fresh tissue was cut in small pieces and ground in a mortar with fine sterile sand. 3 cc. of glycerin and 30 cc. of sterile alkaline 0.9 per cent saline (NaOH 1-1000) were added to each 10 gm. of tissue and the mass thoroughly triturated. The mixture was washed once with ether to remove any fat present and then centrifuged. After removal of the ether the supernatant fluid served as a test solution for inhibitors. A stock tumor was taken about 3 weeks after grafting and cut into generous pieces which

were notched in several places to allow a greater surface area to come in contact with the solution. These grafts were allowed to soak in the extract for 15 to 20 minutes. They were then injected into the groin of the test animals by means of a trocar. A fraction of a cubic centimeter of the extract would be injected with the graft. At the same time grafts were cut from the same tumor and allowed to soak for the same period of time in alkaline saline and glycerin of the same concentration

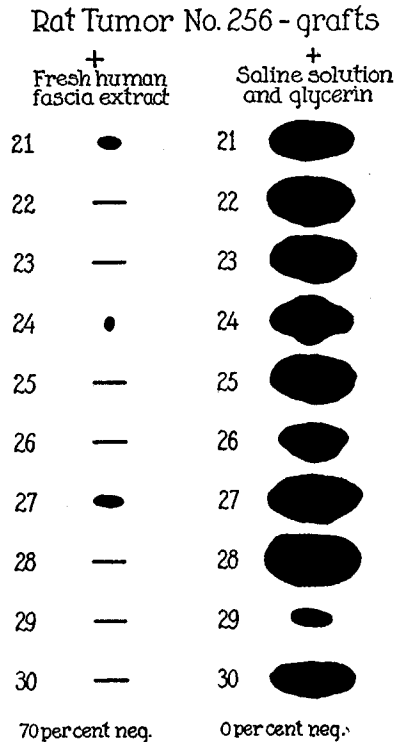


FIG. 2. Almost complete inhibition of the tumor growth was obtained in three such experiments. The silhouettes are one-third the size of the growths.

used in the extraction process. These grafts were similarly injected into the groins of control animals. Precautions for asepsis were used throughout.

#### EXPERIMENTAL

The outcome of a typical experiment is shown in Fig. 1. The silhouettes on the right taken from measurements made 5 weeks after inoculation indicate that in 90 per cent of the control animals the

tumor had attained an average size of 3.0 x 1.5 cm. whereas the fresh human fascia extract had caused a complete inhibition in the case of the others. This identical result was attained in three separate experiments. In three other experiments results as indicated in Fig. 2 were noted. The growth in the control series behaved as usual but

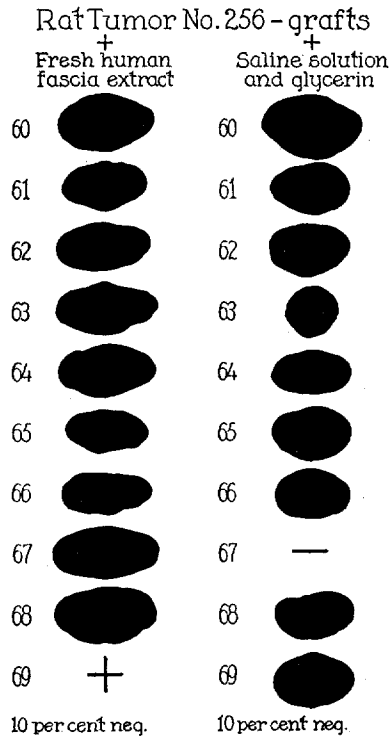


FIG. 3. The connective tissue extract caused decided retardation of the tumors for 3 weeks. After that period the retarded tumors grew faster than the controls. This occurred in one experiment only. The silhouettes are one-third the size of the growths.

there was not complete inhibition of all of the test tumors, though a definite retardation was noted in all cases. In two of these experiments three small tumors grew in the test animals, and in the third experiment five such tumors appeared. In a seventh experiment the controls grew as usual, but although there was a definite slowing and

TABLE I

Material inoculated with Rat Tumor 256	Experiment No.	No. of test rats inoculated	No. of test tumors at 5 wks.	Average size at 5 wks. <i>cm.</i>	No. of controls inoculated	No. of tumors at 5 wks.	Average size at 5 wks. <i>cm.</i>
Fresh human rectus sheath	1	10	0	—	10	9	3.13 x 1.75
	2	10	0	—	10	9	3.20 x 1.54
	3	10	5	1.2 x 0.42	10	10	3.16 x 2.01
	4	10	3	0.83 x 0.46	10	10	3.18 x 1.64
	5	10	0	—	10	9	3.04 x 1.72
	6	10	3	0.92 x 0.43	10	8	3.15 x 1.96
	7	15	14	3.75 x 1.95	15	14	3.05 x 2.02
Totals.....		75	25		75	69	

Rat Tumor No. 256 - grafts

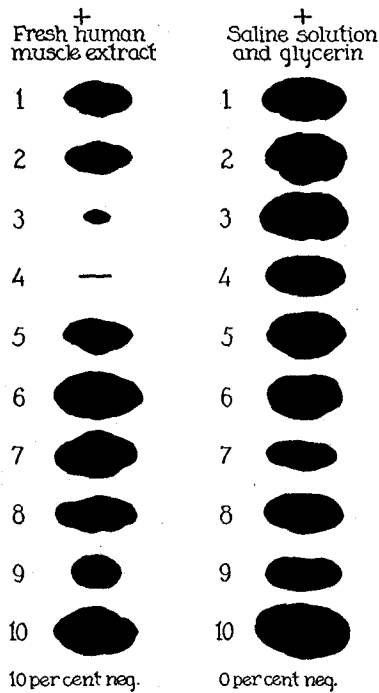


FIG. 4. Muscle tissue extract had practically no effect. The silhouettes are one-third the size of the growths.

inhibition to the 3rd week, 14 of the 15 test tumors not only grew but actually seemed to grow faster than the controls when once the inhibition had been overcome. At the end of the 5 weeks period these test tumors measured slightly more than the controls. This is shown in Fig. 3. The results of all these experiments are listed in Table I.

In contrast to the action of extracts of connective tissue we have presented in Fig. 4 the outcome of a typical experiment with extracts of human muscle, in which exactly the same method of preparation was utilized. Here any effect noted was well within the normal variation in behavior of the tumor.

#### DISCUSSION

These observations are perhaps too few to warrant any sweeping deductions. It is fully realized that much more evidence must be accumulated with other test tumors. This is now being done in our laboratories. The results noted, however, may be taken to indicate trends which can be proved or disproved later. The work of Murphy and his collaborators seems to point to the interaction of two forces inherent in every cell to maintain it in a balanced state. Under normal conditions the balance is perfectly controlled. These regulators in each cell must be small in quantity and easily changed over to some other form. The experiments made by Murphy and his coworkers show that it is possible to extract both stimulators and inhibitors from the tissues. It has seemed to us from long observations on healing wounds that there must be a mutual interplay of forces between tissues derived from different embryological layers. Thus, in every healing wound the balance must be carefully adjusted between the epithelium on the one side and the granulation tissue on the other. It seems probable that embryonic types of connective tissue contain inhibitors for epithelium at certain stages, *i.e.* near the scar-forming period; and that earlier in the red active stage the epithelium is stimulated by the granulation tissue. It was for this reason that we decided to try extracts of the more undifferentiated type of connective tissue against an epithelial tumor. Rat Tumor 256 has been described as a pure carcinoma which "takes" in almost 100 per cent of transplants, and hence it was selected as the test object. Perhaps this selection has been unfortunate since it has been

found on further investigation that not all authorities regard Rat Tumor 256 as a pure carcinoma. By some it is held to be a carcinosarcoma. It has also been observed to grow in only 75 per cent of transplants in some laboratories. The experience of several workers in this University has been that it grows in about 90 per cent of transplants. There have been periods of growth fluctuation when it has receded, but the growth of the control grafts indicates that these experiments were not conducted at such a period. The extracts of connective tissue were not desiccated in our observations, another difference from Murphy's experiments. In future experiments, this will be done as it apparently enhances the potency of the inhibiting factor. Extracts of muscle—a highly differentiated form of connective tissue—were not effective against the growth of transplants of Rat Tumor 256.

Objection may be raised to these experiments in that the inhibitor substance came from a heterologous source. In answer to this, there was not observed any toxic reaction or condition which might be regarded as allergic. Furthermore, the failure of human muscle extracts prepared in the same way to influence the tumor growth serves as adequate control to this point. Homologous rat fascia is so thin that it does not lend itself to trial. In Murphy and Sturm's most recent papers the inhibiting effects from some heterologous tissues were quite definite.

A few attempts to cause recession of established transplants of Rat Tumor 256 by intraperitoneal and subcutaneous inoculations were not successful. This phase of the problem must be deferred for the present.

#### SUMMARY

Extracts from fresh normal human connective tissue (rectus sheath) exhibited a decided inhibiting action on grafts of Rat Tumor 256 transplanted into test animals. There was complete inhibition of tumor growth in 66 per cent of the animals; there was a marked retardation in the rate of growth in another 15 per cent, the tumors in these instances being much smaller than the controls transplanted at the same time. In only one experiment was there failure to obtain a lasting growth-inhibitory effect. The tumors in these animals (19

per cent) although showing an initial retardation in growth, apparently overcame the restraint and at the end of 5 weeks were larger than the control series. Extracts from fresh normal human muscle tissue, on the other hand, showed no such inhibiting action on grafts of Rat Tumor 256.

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