9. *Quantitative Expression of Tumor Inoculum.*

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Tumor transplantation is performed by introducing tumor graft into the subcutaneous tissue through an incision in the skin. Occasionally inoculate intraperitoneally or organ. The technic of inoculation may be classed according to the treatment of tumor material implanted as 1) Tumor piece transplantation, 2) Tumor gruel inoculation and 3) Tumor cell suspension injection. Subsequent development of the implant depends on the strain, age and nutrition of the host animal, the site of inoculation and the character and quantity of the inoculum. The tumor which were progressively growing and free from necrosis shows rapid growth in the new host, while the one which were remaining stationary or disappearing leads to unsuccessful transplantation.

The quantity of the inoculum is a very important factor which affects direct influence upon the taking and grows of the tumor. Upon surveying the works dealing with tumor transplantation very few attempt to mention bearing of the dose of implants correctly. The object of this paper is to review the quantitative expression of tumor inoculum offered by Sasaki⁶⁾ in 1943, which is practically significant in experimental studies on tumor transplantation. It will not be without interest to recall how investigators express the quantity of inoculum. A brief statement about the expression in respective technic will be given in the following.

1. **Tumor piece transplantation.** In this technic a small piece of tumor tissue is inserted by the aid of fine forceps or a fine trocar. Some investigators express the dose by the size of tumor fragment measured by sight as the size of lentil, rice-corn, or millet, while others by the weight in mg. In the heterotransplantation of Ehrlich mouse carcinoma to white rat, Putnoky¹⁾ utilized the canule technic (technic after Murray-London), by which

he introduced fragments of the size of a lentil weighing 300–400 mg.

2. Tumor gruel inoculation. Tumor gruel is prepared by cutting tumor tissue finely with scissors in a flat stender. Inoculation is effected by means of a syringe provided with a needle of about 1.5 m.m. diameter or a trocar of suitable size. In syringe technic the dose is expressed by reading the graduation in c.c. on the syringe. In trocar technic the most investigators do not customally express the dose quantitatively. Usually they record only the diameter of trocar in m.m. Yano²⁾, in one series of his experiments, tried to inoculate cancer gruel of Bashford mouse carcinoma by trocar technic. The dose he inoculated is unknown.

3. Tumor cell suspension injection. The tumor cell suspension is prepared by putting an amount of tumor gruel in a mortar and adding necessary quantity of physiological saline solution to it, mix them grinding, and filter the fluid through lint on a funnel to remove clumps of tissue fragment. The filtered fluid is a suspension of finely dissociated tumor. Injection is effected by the aid of an ordinary syringe. The dose is expressed by the rate of saline added and the amount of suspension injected in c.c. Yano²⁾ injected subcutaneously a suspension 0.2 c.c. in each mouse by means of a 1/1 m.m. needle attached to a Luetin syringe. The suspension was prepared by diluting gruel with a saline up to ten times its weight, ground and filtered through a sheet of sterile lint.

Cellular amount in per cent contained in a suspension may be estimated by centrifuge method. Schrek³⁾ determined the percentage of centrifugable solids in the suspensions in the following manner. A Bauer-Schenck tube containing 0.5 c.c. of the suspension was centrifuged at high speed for fifteen minutes and the amount of precipitated solid was estimated. Most of the suspensions examined had about 50 per cent solid. He inoculated the suspension, undiluted and diluted 1:10 and 1:100 with physiological saline so as to the dose correspond to 0.1 c.c., 0.01 c.c., 0.001 c.c., and 0.0001 c.c. of the original suspension.

4. Injection of tumor suspension containing small amount of solids. In the case of tumor suspensions containing small amount of solids, the dose of inoculation is expressed by the number of tumor cells. By employing hemocytometer, count the number of

cells suspended in a standard suspension. To obtain a suspension containing a known number of cells, dilute the standard suspension by adding saline. By the above method, Costa⁴⁾ showed that the grafts will not take and grow if the number of cells in 1 c.c. suspension inoculated falls below 1,000,000 in Ehrlich mouse carcinoma and 100,000 in mouse sarcoma 37. De Gäetani⁵⁾ has set the minimum number which could be counted on to elicit the mouse sarcoma 37 was 430,000, if there were suspended in 1 c.c. of physiological salt solution. But when the deposits from centrifuged suspensions were employed, 100,000 cells repeatedly gave rise to vigorous growths.

The piece or gruel technic is preferable in general and more satisfactory results may be expected than the suspension technic. Because in the latter the practical amount of tumor material is smaller and is subjected to more mechanical and chemical hindrance than the former. When the tumor is hardly transplantable such as in the case of successive transplantation of a spontaneous tumor or in heterologous transplantation, the piece or gruel technic should be employed. The suspension technic is usually applied to easy transplantable tumors.

Among technical factors the quantity of the inoculum is the most important factor influencing the takes and growths of the tumor. A tumor strain which is used for experiments in laboratory should always be kept in good situation applying successive transplantation. For this purpose it is necessary to inoculate every passage with an optimal dose raising growths in high percentage. It is valuable to record correctly the optimal dose of each transplantable tumor. From this point of view Sasaki⁶⁾ presented a form of quantitative expression to show the quantity of single optimal dose of inoculum in terms of average values, at least in ten times, of its weight, moisture and dry matter in mg. It is applicable to all technics except 4 above mentioned. Following the Sasaki's suggestion the author estimated the weight of single gruel inoculum optimal for Bashford mouse carcinoma.

A fine trocar was used. It is constructed of two parts, a trocar and a needle attached to a haft. Whole length of it 105 m.m., the trocar 34.5 m.m. in length and 1.6 m.m. inner diameter, the needle 43.5 m.m. in length and just pass through the trocar. To fill up

the trocar with tumor gruel, let the gruel draw in through the tip of the trocar by pulling out the needle gradually. By this manipulation the inner space, 32 m.m. in length and 1.6 m.m. diameter, is filled up with a dose of single inoculum for mouse. The weight was estimated according to rule by putting one dose in every ten weighing bottle. The figures in Table 1 give the weights of the gruel prepared with a well developed ten days' tumor. The average in each column is shown in the last row; the weight 66.9 mg., moisture 56.9 mg. and dry matter 10.0 mg. For the sake of simplicity it was assumed that the above inoculum as a unit dose of gruel of Bashford mouse carcinoma.

Table 1. The weights of 1 unit tumor gruel of Bashford mouse carcinoma.

No. of weighing bottle	Weight mg.	Moisture mg.	Dry matter mg.
1	64.2	54.6	9.6
2	67.7	57.6	10.1
3	64.9	55.3	9.6
4	68.4	58.5	9.9
5	70.6	59.8	10.8
6	70.2	59.6	10.6
7	65.3	55.7	9.6
8	64.9	55.6	9.3
9	66.8	56.8	10.2
10	65.8	55.6	10.2
Average	66.9	56.9	10.0

As the host animal 10 20 g. healthy white mouse was used. The inoculation was performed with sterile precautions by inserting the trocar into the subcutaneous tissue of the dextral abdominal region through an incision on the dextral thoracic region of the skin. Push the needle passing through the trocar and draw out the trocar simultaneously so as to the gruel is inoculated linearly and diffusely and not to form a mass. In ten to twelve days after inoculation of this unit inoculum the tumor is transplanted to the next passage. If a tumor was chosen, which at that time gave rise to the growth of the size of the tip of little finger, weighing about 1.5 to 2.0 g., soft, parenchymatous and non-necrotic tumor material with a high growth energy is obtained and almost entire

tissue may be utilized for inoculation. On the other hand tumors which have passed two weeks or more after implantation the necrotic region in centre is enlarged and the tissue becomes fibrous. In these tumors only the peripheral region is available for inoculation. By a series of tests on the keeping of the tumor strain in good condition it has been proved preferable to transplant successively in about ten days.

Table 2. A comparison of the average weights of a unit tumor gruel following the number of days passed after inoculation.

Days passed after inoculation	Weight mg.	Moisture mg.	Dry matter mg.	Moisture per cent	Dry matter per cent
10	66.9	56.9	10.0	82.1	14.9
15	70.4	60.5	9.9	84.4	14.1
30	66.8	57.8	8.9	86.5	13.3

Table 2 gives a comparison of the average weights of the unit gruel prepared with the tumor which has passed ten, fifteen and thirty days after inoculation. Figures in the fifth column are the percentages of moisture and the sixth are the percentages of dry matter. It shows the tendency increase in moisture and decrease in dry matter. That in the same quantity of unit inoculum such differences appear is probably due to the degree of histological degeneration of tumor tissue following the number of days passed after inoculation. Attention should be called to the fact that by the above stated quantitative expression not only the quantity of the inoculum is correctly shown but also may indicate the histological character of the tumor material implanted which has an effect to the percentage of takes and growth of the tumor.

Literature.

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