ON THE POSSIBILITY OF TRANSPLANTING LYMPHOBLASTOMA AT DIFFERENT INTERVALS AFTER THE HOST'S DEATH

Z. A. Butenko and M. K. Dal

From the Department of Pathologic Physiology (Head - Prof. O. A. Bogomolets) and from the Department of Pathologic Anatomy (Head - Prof. M. K. Dal), the Kiev Postgraduate Medical Institute (Director - Prof. L. I. Kalchenko)

(Received November 24, 1956. Presented by Prof. A. D. Timofeevsky, Active Member of the USSR Academy of Medical Sciences)

It is known that experimental tumors and leukosis can be reproduced by using implantations of blastomatous or tumorous tissue from animals just killed [1, 2, 3, 4, 5, 6, 7 and others]. We could find no indication in the literature as to the time limit for positive results from the given transplantations. This question is important for two reasons: first, because it is not always possible to obtain tissue for transplantation immediately after the patient's death, and second, because it would be interesting to know how long after the death of the host the blastomatous and leukotic cells retain their vitality.

EXPERIMENTAL METHODS

We produced an implantation of LIO lymphoblastomatous tissue by means of approximately 80 passages of the tissue to a highly-leukotic strain (Afb) of white mice. Five mice with a more or less generalized lymphoblastoma were used as the donors. The number of leukocytes in the peripheral blood of the animals was 32,200, 35,020, 36,400, 50,800 and 60,000 per 1 mm^3. We found lymphoblasts (up to 19%), prolymphocytes and single normoblasts in the blood smears.

The animals were killed by ether narcosis; their bodies were kept at room temperature (about + 18°), and several transplantations were made from each animal. Under sterile conditions, pieces of tissue were taken from the enlarged spleen, the lymph nodes and subcutaneous tumorous growths of the animal directly after its death and then after 6, 12, 24, 30, 48, 60 and 75 hours. A suspension was prepared from the tissue pieces in a physiological solution; 75,000-100,000 cells per 1 mm^3 were contained in the suspension, which was then injected either subcutaneously or intra-abdominally into healthy white mice of the Afb. strain, 1-1 ½ months old, in a dose of 0.3 cc.

At the same intervals after the death of the animals, pieces were also resected from the organs and tissues which had been used in the preparation of the suspension and also from the liver, kidneys and lungs to be used for microscopic examination, and impression preparations were made from the lymphoblastomatous nodes. The dried pieces were fixed in 10% neutral formalin and embedded in celloidin. The sections were stained with hematoxylin-eosin by Van Gieson's method and were impregnated with silver nitrate according to Gomori.

The transplantation suspension was proven sterile in selective bacteriological examinations.

The blood of the mouse recipients was examined at different intervals after the beginning of the experiment (after 7, 10, 12 days, and every 5 days thereafter in longer experiments). After the death of the animals, a pathologico-anatomical autopsy was done, and the pieces of tissue were microscopically examined.

In all, 82 mice and 8 different periods after the death of the host were experimented with.

EXPERIMENTAL RESULTS

The experiments conducted showed that, to obtain positive implantations, one should consider the time limit of keeping the dead animals at room temperature to be 60 hours (see Table). There was apparently some technical error made in the experiments with lymphoblastoma transplantation 24 hours after the death of the donor mice, which explains the small number (2) of positive implantations. The number of successful implantations began to decrease in the 30-hour experiments. There were negative results after the 2-3 month observation period in the mice which had received material taken 75 hours after the death of the donor mice.

The first palpable development of the subcutaneously implanted lymphoblastoma in the animals appeared on the 7th-10th day; in experiments with transplantation tissue taken at longer intervals after the death of the donor (48-60 hours), the transplanted lymphoblastoma began to grow at slightly later dates, but the tumorous tissue then quickly spread, and the mice died at the same intervals as in the other experiments.

TABLE

Results of Lymphoblastoma Tissue Suspension Implantations

Time of transplantation after animals death (in hrs.)	No. of mice in experiment	No. of positive implantations	Time animals died after implantations (in days)
Immediately	12	11	17—30
6	8	7	17—41
12	8	8	14—37
24	8	2	14—37
30	10	7	15—33
48	15	8	22—35
60	10	4	19—28
75	11	—	—

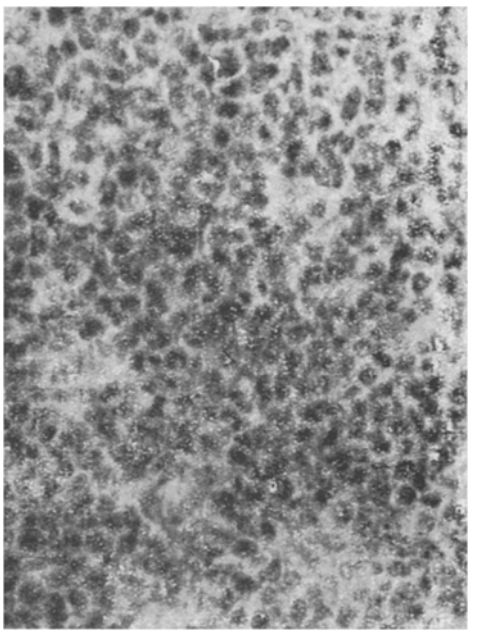

Figure 1. Histological structure of the original transplanted lymphoblastoma.

In 29 of the selectively examined recipient animals with a definite growth of tumorous tissue, the number of leukocytes found in the blood was from 30,000 to 65,000 per 1 mm^3, and there was a predominance of lymphoblasts, often in a condition of karyokinetic division. In 18 of the animals, the number of leukocytes was 11,000 to 26,000 per 1 mm^3, and monocytic cellular elements predominated in the blood.

When the donor animals were dissected, a generalized, subcutaneous lymphoblastoma with leukotic changes in the internal organs was found. In the recipient mice which died, in the subcutaneous cellular tissue at the place of implantation, there were tumorous nodes as large as 3-4 x 2 cm consisting of rather soft, pale pink, homogeneous tissue. Where the lymphoblastoma tissue suspension had been injected intra-abdominally, the same type of tumorous formations as appeared with the subcutaneous injection were found in the mesenteric roots and in the anterior mediastinum. The lymph nodes in these animals were enlarged 2-3 times; in the experiments with longer intervals after the death of the animal before the preparation of the lymphoblastomatous tissue suspension, the lymph nodes in the neck were especially large. The spleen was 3 times it normal size.

When the tumors in the donor and recipient mice were microscopically examined, typical lymphoblastoma was found: the tumor cells were homogeneous, large, with a round nucleus, did not stain intensely and had a small quantity of homogeneous protoplasm. The cells were closely packed and many instances of karyokinetic division were found among them (Fig. 1). Capillaries were found occasionally; the argyrophilic fibers were thin and sparse. Almost the entire tissue of the lymph nodes and spleen had been replaced with such tissue. There were small perivascular accumulations of lymphoblasts in the liver and, in some animals, in the kidneys and

peribronchially in the lung tissue. We found a large number of large and small lymphoblast cells with well-stained nuclei on the impression preparations.

Six to twelve hours after the death of the donor mice, microscopic examimination showed signs of post - humous decay in the liver and kidneys. There were no longer any dividing cells in the lymphoblastoma nodes; the nuclei of the lymphoblasts stained more intensely than formerly, and in some of the cellular elements, fragmentation of the nuclei was observed. These changes gradually intensified, and 48 hours after the death of the animal donors, nuclei of different sizes were observed in the lymphoblasts, many of them as large as lymphocytes and intensely stained; many of the cell nuclei disintegrated into large lumps. However, typical lymphoblasts were still found among the changed cellular elements (Fig. 2). The argyrophilic fibers were intensely impregnated with silver nitrate. Swollen hepatic parenchyma cells were observed and the nephritic epithelium, especially in the convoluted tubules, was swollen, with cloudy protoplasm and nuclei which were not always clearly stained.

Sixty hours after the death of the animals, the disintegration of the cell nuclei was sharply expressed in the peripheral sections of the tumorous nodes; in places, they were not stained at all. In the other sections, the lymphoblastoma tissue had greatly disintegrated; the cells were polymorphous with, for the most part, small nuclei, frequently angular in shape, diffusely and intensely stained; extremely numerous, small granules of chromatin were found, and typical lymphoblasts could still be seen, but not in every microscopic field (Fig. 3). The argyrophilic fibers were partially fused and in a state of fragmentation. Detritus from the decaying cells, granules of chromatin and occasionally comparatively well-preserved lymphoblasts were observed on the impression preparations. Decay was sharply expressed in the liver and kidney tissue— many cellular elements did not stain. Macroscopically, the changes caused by posthumous decay were very apparent, especially in the gastrointestinal tract.

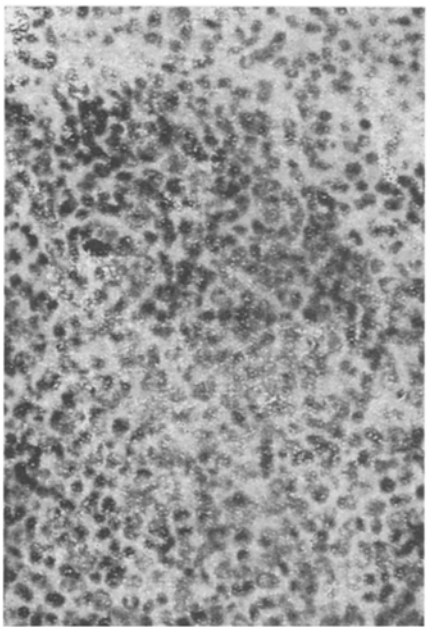

Figure 2. Histological structure of lymphoblastoma 48 hours after the animal's death.

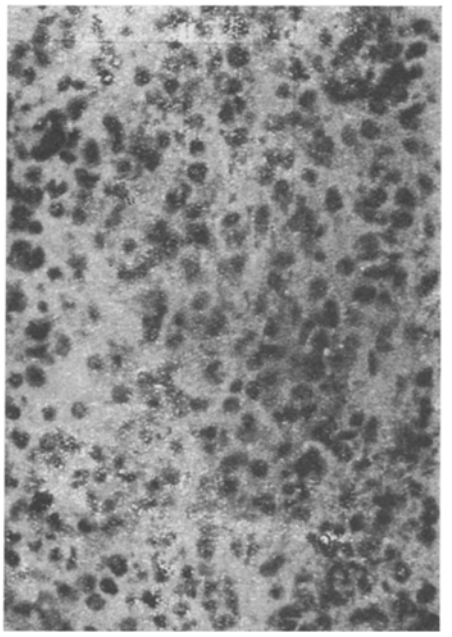

Figure 3. Histological structure of lymphoblastoma 60 hours after the animal's death.

Seventy-five hours after the animals' death, organic decay was acute. The cellular elements in the liver, kidneys and lungs did not stain. No preserved cells could be found in the tumorous nodes; the tissue consisted almost entirely of detritus with many small clumps of chromatin.

Generally, one can say that there was a parallelism between the number of positive lymphoblastoma implantations to the recipient mice and the histo-morphological picture of the transplant tissue at different times after the death of the donor animals.

From the experiments conducted, one can conclude that transplantations can succeed up to 60 hours after the death of the host animals, even when their bodies are kept at ordinary room temperature. At this time, most of the cellular elements of the transplant tissue are in a state of decay, and only individual lymphoblastic cellular elements are still preserved. These elements are apparently more resistant to the process of posthumous decay than the tissue of the liver and kidneys.

SUMMARY

Transplantation of cellular suspensions of lymphoblastoma, kept at 18°C is successful if performed not later than 60 hours after the host has died. The number of successful transplantations decline if performed 30 hours after the death of the host. No transplantation has ever been successful if made 75 hours after the host's death. Microscopic studies made of later transplantations revealed a large amount of detritus in the implanted tissue.

LITERATURE CITED

[1] Petrov N. N., A Brief Synopsis of the Data Basic to the Development of a Theory on Tumorous Growth (in Russian), 1954.

[2] Raushenbakh M. O., Experimental Studies of Leukoses (in Russian), 1956.

[3] Shabad L. M., Essays in Experimental Oncology (in Russian), 1949.

[4] Shabad L. M., Experimental Methods of Producing Tumors and Leukoses, in the book: The Reproduction of Disease in Animals for Experimental Therapy Purposes (in Russian), 1954.

[5] Yudina N. D., Hemoblastoses (Doctorate Dissertation in Russian), 1940.

[6] Engelbreth-Holm., Spontaneous and Experimental Leukemia in Animals, 1942.

[7] Furth L., Recent Experimental Studies on Leukemia, 1946, v. 26, No. 1.

[8] Furth L., Seibold N. R., Rathbone R. K. Experim. Studies on Lymphomatosis of Mice Amer. J. of Cancer., 1933, v. 26, N. 3.