A METHOD FOR COUNTING THE VIABLE CELLS IN NORMAL AND IN MALIGNANT CELL SUSPENSIONS

ROBERT SCHREK, M.D.

(From the Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee)

Pappenheimer (1) developed a simple method by which, with the diffusion of trypan blue into the nucleus as a criterion of cell injury, he was able to study quantitatively the effect of various agents upon cell suspensions of the thymus. Trypan blue was added to suspensions prepared by teasing apart freshly excised thymus tissue. The dye stained readily the injured cells, but did not stain the uninjured cells. The differentiation of stained and unstained cells was usually marked. Under certain conditions, however, various degrees of transitional staining were encountered which made some of the determinations uncertain. Pappenheimer was able, by determining the percentage of stained cells, to demonstrate the injurious effects of various reagents (cytotoxic immune serum, acid) on lymphocytes. Richter and MacDowell (2) used Pappenheimer's method for studying the injurious effects of physical and chemical agents on leukemia cells.

Studies (3) of the effect of toxins on tumor cells brought out the need for a method of determining the number of viable cells in normal and in malignant cell suspensions. Shear (4), studying the titration of tumor cell suspensions, also pointed out the necessity of knowing the number of viable cells used for inoculation.

The resistance of viable cells to staining affords an opportunity of differentiating viable from non-viable cells. This report presents details of the method used in this laboratory. The method has been applied to preliminary studies of the effect of deleterious agents (moccasin venom, ricin, high temperature) on tumor cells.

METHODS

Suspensions of organs and tumors were prepared by grinding the normal or malignant tissue in a Latapie apparatus and filtering the suspension through 80 mesh Monel metal wire cloth. Complete details of the method used for the preparation of suspensions are given in a previous paper (5).

To 0.2 c.c. of suspension was added 4.8 c.c. of a 1:2000 eosin solution in Tyrode's at pH 7.6. The mixture was shaken for two minutes and a drop was then placed in a hemocytometer. Stained and unstained cells could be readily differentiated. The stained cells were diffusely pink. The unstained cells were transparent or were slightly yellowish as compared to the stained cells. The unstained cells in one or more square millimeter fields were counted, and the number per cubic millimeter was calculated.

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Methylene blue in concentrations of 1:2000 was used in some experiments. This dye also gives satisfactory differentiation of stained and unstained cells, but the differentiation is not as good as with eosin. Methylene blue has the advantage, however, of staining clearly the nuclei of the non-viable cells.

Each dye solution, before being used, was tested for its capacity to stain rapidly and uniformly all the cells in killed suspensions, and for its capacity to provide sharp differentiation of stained and unstained cells in fresh suspensions.

**RESULTS**

*Walker Carcinoma 256:* Suspensions of tumor 256, prepared by the above methods and stained with eosin, were found on microscopic examination to consist of numerous isolated tumor cells, a few small clumps and occasional blood cells. A large percentage of the tumor cells were stained pink or red by the eosin. The cells were large and round or irregular. When methylene blue was used, the nucleus of the stained tumor cell was found to be large and round, with a conspicuous nuclear membrane, a large nucleolus, and a small number of chromatin granules. A few nuclei were in mitotic division. The unstained cells were sometimes flattened, hyaline, and almost transparent. More usually the unstained cells were spherical with a yellowish sheen which contrasted markedly with the pink eosin-stained cells. In suspensions that had been kept for a few days in the refrigerator, the unstained cells were filled with large clear vacuoles.

The cell suspensions of 10 Walker tumors were counted. The number of viable cells varied from 3000 to 18,500 cells per cubic millimeter. The percentage of viable cells was usually about 10 per cent.

*R39 Sarcoma:* Cell suspensions of the R39 tumor were found to contain, after the addition of eosin, numerous, large, unstained cells and a small number of unstained leukocytes and erythrocytes. Two cell suspensions of R39 sarcoma were counted and were found to contain 19,500 and 57,800 viable cells per cubic millimeter.

*Normal Organs:* Suspensions were also made of the liver, kidney, and testis of rats. The liver and kidney gave rise to suspensions composed of numerous small clumps of cells, few isolated parenchymal cells, and many leukocytes and erythrocytes. These suspensions were not suitable for counting. Satisfactory counts could be made with the suspensions of the testis as these suspensions contained numerous isolated small and large round cells, numerous spermatocytes, and a few blood cells. Very few of the cells were stained or in clumps. The spermatocytes were not motile at room temperature. Spermatocytes which had their tails broken off were stained by the eosin. One suspension was found to contain 330,000 unstained round cells per cubic millimeter.

*Effect of Toxins and High Temperature on Tumor Cells:* The viable-cell count method described above yielded interesting information on the effect of various injurious agents on tumor cells. Toxins differed in their action towards Walker tumor cells. In one experiment, moccasin venom, 1:200, was added to an equal volume of tumor cell suspension. After two minutes, eosin was added and the mixture examined microscopically. No unstained cells were found. In an analogous experiment, ricin, 1:200, was added to an-
other Walker tumor cell suspension. The number of viable cells in this mixture was determined in two minutes and at the end of an hour. The ricin, in contrast to the venom, did not cause any decrease in the number of viable tumor cells. It appears then that moccasin venom has an immediate deleterious action on Walker tumor cells while ricin did not injure the cells during an observation period of one hour. This finding may be helpful in explaining the long latent period of ricin and the absence of a latent period for the venom when these toxins are injected intradermally.

The effect of high temperatures on Walker tumor cell suspensions was of interest in view of the numerous attempts to treat cancer by raising the temperature of the body. A suspension of tumor cells was kept at $43^\circ$ C. and duplicate viable cell counts were made on the suspension at intervals. The results are given in Fig. 1. The figure shows that at $43^\circ$ C. the number of viable tumor cells decreased slowly. Some tumor cells were viable even after four hours of exposure at $43^\circ$ C. Further studies are planned to compare the resistance of malignant and normal cells to heat.

**Summary**

Cell suspensions of Walker tumor 256, R39 sarcoma, and rat testis were obtained by grinding the tissue in a Latapie apparatus and filtering through 80 mesh Monel metal wire cloth. The suspensions were diluted 25 times with 1:2000 eosin in Tyrode's at pH 7.6. The unstained cells were counted by use of a hemocytometer and the number of unstained cells per cubic millimeter was calculated.

Moccasin venom (1:200) was found to have an immediate deleterious action on Walker tumor cells. Ricin (1:200) appeared to have no immediate injurious action on Walker tumor cells, as indicated by viable cell counts. Exposure of a Walker tumor cell suspension to $43^\circ$ C. caused a slow decrease in the number of viable cells.
REFERENCES


