METHODS

THE TECHNIQUE OF THE ONCOLYSIS REACTION

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In 1910, Freund and Kaminer [2] found that the serum of healthy human subjects and animals possesses the power of dissolving tumor cells in vitro, whereas the serum of animals and human patients suffering from cancer has lost this property.

Attempts have been made to use this phenomenon of oncolysis or cancerolysis, for diagnostic purposes. It was found, however, that loss of the oncolytic power of the sera was not specific for cancer. This must not, however, be regarded as an obstable to the use of the oncolysis reaction in oncological practice. In particular it is of interest to study the changes in the oncolytic activity of serum as a result of the action of antitumor drugs. If in the course of treatment by these drugs, the oncolytic activity of the sera of cancer patients was restored, then this could be used as an indication of the effectiveness of the treatment.

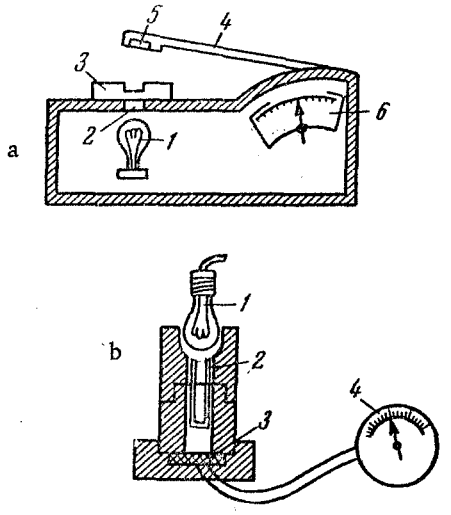

Fig. 1. An outline of the construction of the densitometers. a) Densitometer EEL; b) densitometer as constructed by the author.

The wide use of the oncolysis reaction is also prevented by defects in the method of performance of the test which has so far been most frequently adopted, based on counting the visibly unchanged cells before and after contact with the serum [2]. The first experiments which we performed by this method did not give comparable results. Attempts to develop a more accurate and less laborious technique have not yet been successful.

The aim of the present work was to develop a method of performance of the oncolysis reaction which would give standard results and which would, at the same time, be less laborious than the method of counting the tumor cells.

We decided to use the principle of photometry, starting from the following assumption. If, as a result of the oncolytic activity of the serum, the number of tumor cells in the given medium is reduced, the optical density of the medium must also be reduced correspondingly. The change in the intensity of a beam of light after passing through such a medium, which reflects the degree of lysis of the tumor cells, is easily measured.

For the experiments we used the "Densitometer EEL" (Fig. 1, a). Over the aperture 2, situated directly above the light source 1, is placed the vessel 3 containing the medium whose optical density it is required to measure. On

the vessel rests a handle 4, with a photoelectric cell 5, connected to a galvanometer 6; the scale of the latter is graduated in units of density. The vessel used in measuring the optical density of the medium was a specially made plexiglas plate in which were flat-bottomed hollows measuring 6 mm in diameter and 6.5 mm in depth. In addition to this, we constructed an apparatus whose action was based on the same principle (Fig. 1, b). On to a base is fixed a screw-type lampholder with a small aperture in the center. At the base of the lampholder is mounted a photoelectric cell 3. Above the aperture is placed the light source 1 (the illuminator of an OI-7 microscope). Inside the lampholder is placed a flat-bottomed tube 2, 2.5 mm in diameter, with the medium for testing. The photoelectric cell is connected to a mirror galvanometer 4. A beam of light, passing from above through the tube containing the fluid for testing, and thereby changing in intensity, falls on the photoelectric cell connected to the galvanometer. The galvanometer readings are used for calculation of the oncolytic index.

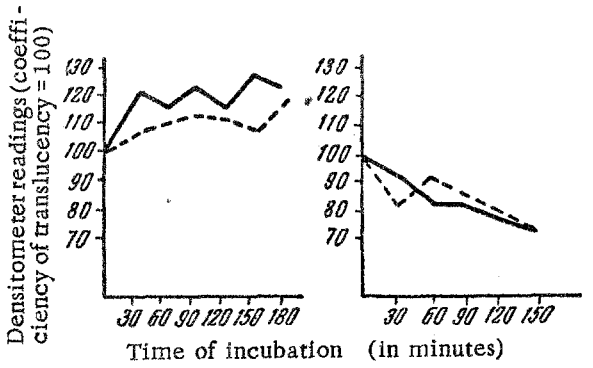

Fig. 2. Typical curves of the optical density of a suspension of tumor cells during incubation at 37°.

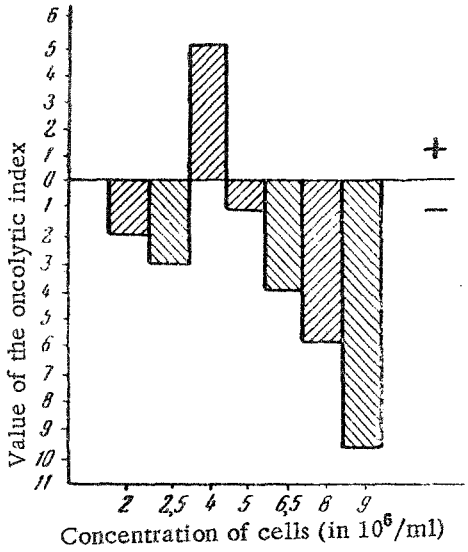

Fig. 3. Determination of the optical concentration of the suspension of tumor cells.

Measurement of the optical density of the medium contained in the hollows or tubes was carried out without delay after addition of the serum to the suspension of tumor cells, and then after different intervals of time through which the two were in contact with each other at 37° (equal volumes of serum and tumor-cell suspension were used). A portion of the tumor-cell suspension, added to Freund's solution, acted as a control. The tumor-cell suspension was prepared in the usual way [2]. The oncolytic index (OI) was calculated from the formula:

$$OI = \frac{OK_1}{O_1K},$$

where O and O_1 were the densitometer readings in the experiment before and after incubation respectively; K and K_1 were the control densitometer readings before and after incubation respectively.

The index was expressed by the number of hundredths by which the value exceeded unity or was less than unity. In the former, the index was preceded by a + sign, in the latter by a − sign. For example, 1.09 was expressed as +9; 0.88 as −12. This method of expression of the indices was adopted because in estimation of the oncolytic activity of the sera by the method of photometry, the absolute number of hundredths was of fundamental importance in our experiments.

In order to determine the optimal time of contact of the tumor cells with the serum, suspensions of tumor cells with serum (and also a control) were incubated for 30, 60, 90, 120, 150 and 180 minutes. The optical density was measured before and after incubation of the suspensions. From the results obtained, curves were drawn: along the abscissa was shown the time of contact, and along the ordinate, the densitometer readings with a coefficient of translucency equal to 100. For construction of the curves the results of experiments repeated not less than three times were selected. Theoretically the control curve should have shown the course of autolysis alone, and the experimental curve the course of autolysis of the cells plus that of specific oncolysis. Both the experimental and control curves should have reflected a continuous decrease in the optical density of the medium. Where the medium to be tested showed oncolytic activity, this decrease should have taken place more rapidly in the experimental test than in the control. The character of the curves was, however, much more complicated. Evidently the course of change in the optical density of the medium was influenced by agglutination, sedimentation and autolysis of the cells. All these factors complicated the curves and made their analysis more difficult. In order to simplify the analysis, model experiments were carried out,

for the purpose of obtaining curves showing changes in the optical density of the medium in cases where agglutination, sedimentation or autolysis of the cells was present in the "purest" form. These experiments greatly simplified the analysis of the complex curves which were obtained as a result ofplotting the results of the series of experiments aimed at estimating the optimal times of incubation of the tumor-cell suspensions in the serum.

Altogether 11 experiments were performed, each of which was repeated from three to five times. In Fig. 2 are shown the curves of two typical experiments, reflecting the course of the change in the optical density of a suspension of tumor cells during incubation. Analysis of the curves showed that in the majority of cases the optimal time of incubation of the cells in serum was 60 minutes. This was also confirmed when the results obtained were tested for standardization. The limits of variation of the results, as a percentage of the mean OI, were least (26.1) in those cases when their standardization was tested after 60 minutes. In all the remaining cases the limits of variation were wider (from 28.1 to 44.6%).

Oncolytic Indices as Determined by the Cell Counting and Photometric Methods

Diagnosis of disease	OI, determined by the method of		Sera of healthy rabbits	OI, determined by the method of	
	cell counting	photometry		cell counting	photometry
Carcinoma of the stomach	0.50	−1	895	0.25	0
Carcinoma of the stomach	0.33	−6	151	1.10	+7
Chorionepithelioma	0.54	−13	152	3.30	+2
Healthy female	2.10	+8	152-A	2.80	+8
Healthy male	2.30	+9	897	1.92	+16

For determination of the optimal concentration of the suspension of tumar cells with the sera, suspensions of tumor cells of various concentrations were mixed. Readings were taken after incubation for 60 minutes. Altogether 10 experiments were performed. Each experiment was repeated not less than three times. The results of these experiments (Fig. 3) showed that the optimal concentration of the tumor cells was 4×10^6 per ml.

In order to find out if the results of the photometric method agreed in principle with those obtained by counting the cells, the oncolytic indices of five sera possessing oncolytic activity and of five sera known to be without such activity, were determined by both methods (see table).

As may be seen from the table, the results of both methods were in principle the same. The sera with no oncolytic activity in both cases gave oncolytic indices below unity, and those with oncolytic activity – above unity.

For a comparative estimation of the value of the two methods we compared the degree of standardization of the results obtained by each. For this purpose 15 experiments were carried out by the method of counting cells, 15 experiments in which the results were obtained by means of the "EEL" densitometer, and 15 experiments with our own densitometer. It was found that by the cell-counting method the limits of variation amounted to 30.39% of the mean value of the OI.

By the photometric method, by the use of the "EEL" densitometer the limits of variation amounted to 12%, and by the use of our own densitometer the limits were 16.94%. These results were treated statistically and were found to be significant ($p = 0.983$).

The information given shows that the method of determination of the oncolytic activity of sera, based on the principle of photometry, is simpler and less laborious than the method of counting cells. Furthermore, the results obtained by the use of the proposed method have a much higher degree of standardization. It may therefore be hoped that the method described may find application in oncology in the determination of the oncolytic properties of sera.

SUMMARY

The proposed method of the sera oncolytic activity determination by densitometers is based on the photometric principle. Optimal conditions for the oncolysis reaction have been established. In principle the photometric method renders results analogous to those obtained by counting the visibly unchanged cells, but is less cumbersome and its results are of a more standard nature.

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