Heat Coagulation of Serum in Cancer: A Method Applicable to Very Small Quantities of Serum

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The method recently described by Huggins, Miller, and Jensen (1949) for measuring defects in the coagulation mechanism of the sera of cancer patients cannot be applied without modification to the sera of small experimental animals because the quantity of serum required is too large. By substituting a measurement of the opacity of the heated serum for a measurement of its coagulability (or inhibition of coagulation by iodoacetate), values which are well correlated with the iodoacetate index of Huggins, Miller, and Jensen can be obtained; these opacity measurements require a very small amount of serum, and are as applicable to the sera of small animals as to that of man.

Method.—0.05 ml. of serum is added to 0.75 ml. of a NaCl-buffer (four parts of 2.2 per cent NaCl plus one part of a mixture of \( \frac{1}{15} \) NaH\(_2\)CO\(_3\) and \( \frac{1}{15} \) Na\(_2\)HCO\(_3\)) at pH 7.0. The mixture, contained in a small test tube, is placed in a boiling water bath for 30 minutes and is then allowed to cool. The extinction coefficient \( E \) of the opaque contents of the tube is found with a microphotometer which has a chamber 1 cm. in depth and has a capacity of about 0.5 ml.; this microphotometer can be constructed by using a microscope tilted horizontally and fitted with a Lange thermopile in place of the eyepiece, together with a potentiometer circuit for measuring the thermopile current. The chamber is fixed on the stage of the microscope in the line of the light path, and the light source is a lamp with a 630 ma filter. The microphotometer is calibrated by inserting neutral filters with known extinction coefficients in the path of the light, the thermopile current corresponding to each filter being measured; this provides a graph from which photometer readings can be translated into extinction coefficients of the opaque diluted serum in the chamber.

The total proteins in the serum are found by a gradient tube density method (2), which requires only a small drop of serum. The extinction coefficient is then divided by the total protein to give the opacity (as a value of \( E \)) per gram of protein, a value which bears a relation to the iodoacetate index.

If larger quantities of serum are available, as in the case of man, 0.5 ml. of serum is diluted with 7.5 ml. of the NaCl-buffer and heated; the opacity can then be determined on a macro scale with any one of the types of photometer commonly found in laboratories.

Correlation with iodoacetate index.—The method in which opacity is used in place of coagulability as measure of a change in the protein pattern of serum does not employ iodoacetate as an inhibitor, although the addition of iodoacetate results in a reduction of opacity, on heating, in much the same way as it results in an interference with the coagulation mechanism. To compare the results with those of the Huggins, Miller, and Jensen method, I have accordingly determined the opacity per gram of protein and the iodoacetate index on 100 human sera, some from normal individuals and some from patients known to have malignant disease. The determinations of the iodoacetate index were carried out exactly as described by Huggins, Miller, and Jensen, except the proteins were found by the CuSO\(_4\) density method instead of by micro-Kjeldahl. The coefficient of correlation between 100 observations of the opacity per gram of protein and the iodoacetate index was found to be 0.79 ± 0.038.1

RESULTS

Tests with small animals.—The results obtained for the opacity per gram of protein in normal and in tumor-bearing rats (Lewis sarcoma 304 and 311, benzpyrene tumors) and in normal and in tumor-bearing mice were carried out exactly as described by Huggins, Miller, and Jensen, except the proteins were found by the CuSO\(_4\) density method instead of by micro-Kjeldahl. The coefficient of correlation between 100 observations of the opacity per gram of protein and the iodoacetate index was found to be 0.79 ± 0.038.1

1 The results obtained with sera having an icterus index of 80 or more are not included. Sera with a high icterus index give unaccountably high opacities after heating, and the correspondence between the opacity per gram of protein and the iodoacetate index is poor. This situation is not improved by using red light or by subtracting the extinction coefficient for the heated, unheated serum.

2 Some of the tumor-bearing rats were obtained from the laboratories of the Rockefeller Institute, through the kindness of Dr. Keith R. Porter. Some of the tumor-bearing mice were obtained from the Roscoe B. Jackson Laboratory at Bar Harbor and some from Rockland Farms, N.Y.
The distinct decrease in the opacity per gram of protein obtained in human sera from persons with various kinds of malignant tumors is also observed in the sera of tumor-bearing rats, but there is no indication of a similar difference between the sera of normal and tumor-bearing mice. The opacity per gram of protein is remarkably variable in the normal mouse.

Experience with the iodoacetate index and the opacity in human cancer.—In the course of the development of this method, considerable experience has accumulated about the significance of the iodoacetate index, as well as that of the reduced opacity of heated serum, in cases of cancer in man. False positives are about as frequent as Huggins, Miller, and Jensen report, and false negatives are more frequent than they indicate. These can occur, on repeated testing, in cases of metastatic carcinoma and even of carcinomatosis; the iodoacetate index and the opacity per gram of protein may accordingly be misleading as diagnostic tests. Another disturbing feature is the large number of “questionable” results, in which a difference between a “positive” and a “negative” test would result from coagulation occurring in adjacent tubes to the one selected. The end point of the coagulation test is none too sharp, and many clinically questionable cases fall into the questionable category from the standpoint of the test. Although the opacity per gram of protein is a figure which has no error attached to it, apart from that of the photometer reading and that of the protein determination, it is not possible to set up a value which will divide all normal human sera from all sera of persons with cancer. Again, the “questionable” class is too large.

The disturbance in the coagulation mechanism and in the opacity-producing mechanism in cancer is nevertheless a real, if not a constant or a primary, phenomenon, and this method of measuring it is described, with an admittedly small number of animals, so that those who have colonies of tumor-bearing animals can make more extensive observations which readily suggest themselves and which can be made when the animals are killed for other purposes. Some of these questions are: in what kind of tumor-bearing animals does the phenomenon occur; at what time during the growth of a tumor does the phenomenon appear; and does it disappear when the tumor is removed?

SUMMARY

A method is described for measuring, as the extinction coefficient per gram of protein, the opacity developed by serum when heated under standard conditions. The values obtained are highly correlated with the iodoacetate index of Huggins, Miller, and Jensen, a decreased opacity corresponding to a decreased coagulability in cancer and other conditions in man. The quantities of serum required are so small that the method can be applied to small experimental animals; a similar decrease in opacity per gram of protein occurs in the tumor-bearing rat but has not been observed in the tumor-bearing mouse, largely because the opacity per gram of protein developed in heated mouse serum varies very widely.

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