Failure To Observe Pentolysis by the Serum of Rats Bearing Malignant Tumors*

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In 1944, Bevilotti (1) reported that the red corpuscles of patients with malignant tumors degrade pentoses in vitro, while the red cells of normal subjects, or of patients with benign tumors, do not have this effect. Menkes (5–8) observed a similar action by blood serum. He found that when the sera of patients with malignant tumors were incubated at 37° C. with pentoses (D-ribose, D-xylose, D-arabinose, or L-xylose) degradation of the pentose occurred with formation of lactic and pyruvic acids. Normal sera and the sera of patients with benign tumors and chronic diseases, such as tuberculosis, cirrhosis, diabetes, and nephritis, did not exhibit this effect. Menkes called this process "pentolysis," since it appeared to be a degradation somewhat similar to glycolysis of hexoses. Menkes considered a degradation of pentose greater than 10 per cent in a 2-hour incubation period a positive diagnosis of cancer. He found an unavoidable error of 2 per cent, and pentolysis values between 5 and 9 per cent were considered doubtful.

Steen (10, 11) carried out studies on the serum of rats in which tumors had been developed by injecting 20-methylcholanthrene. Positive pentolysis tests were obtained in 20 of 22 blood samples from rats bearing experimentally induced tumors. This work supported Menkes' contention that pentolysis produced by blood serum is a manifestation of carcinogenesis.

Kubowitz and Wieding (3) were unable to confirm the postulation of Menkes in experiments with rats bearing the Walker-Karzinen tumor.

The data reported in this paper are the results of a study designed to examine the validity of the so-called pentolysis test of blood serum for the presence of malignancy. During the course of the work, a critical evaluation of analytical methods for the determination of pentoses and furfural was made. Tests were carried out upon the sera of eighteen rats with tumors induced by injecting 20-methylcholanthrene and of ten rats with transplanted tumors.

After this paper had been prepared for publication, a report by Steen (12) appeared in which the author stated "the results of these determinations on a large scale, as well as those of an earlier paper, are thus seen to fall within the limits of the experimental errors of the methods employed for determining the amount of free pentoses. Accordingly, the so-called phenomenon of pentolysis in the blood of rats bearing induced sarcomas is a misnomer."

EXPERIMENTAL

Wistar strain rats, obtained from Carworth Farms, were injected with a single subcutaneous dose of 20-methylcholanthrene (5 mg. in 0.5 ml. of mineral oil) in the abdominal wall. Control animals were injected similarly with mineral oil only. All blood samples were drawn by intracardiac puncture. The blood was allowed to clot, and the serum was removed after centrifugation within 30 minutes. The tests were carried out immediately thereafter. The tumors were removed, fixed in formoldehyde solution, and later examined histologically.

The incubation procedure was identical with that proposed by Menkes (5–8), except that sterile conditions were not maintained. Since the tests for pentose degradation were negative at first and continued to be negative throughout the study, we saw no reason to attempt to eliminate from the digestion mixture micro-organisms that might destroy pentose. Three methods for the determination of pentose were used. These were the procedures of Brachet (2), Roe and Rice (9), and Mejbaum (4). With each of the three methods, pentose was determined in unincubated samples.

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(one tube containing serum and water, the other tube containing serum and ribose) and in samples incubated for 2 hours at 37° C. (serum and water in one tube and serum and ribose in the other). Color was measured by the Evelyn colorimeter, with the use of unincubated and incubated samples of serum and water as control blanks for the unincubated and incubated samples containing serum and ribose. Pentolysis was calculated by dividing the difference in the densities of the unincubated sample and the incubated sample by the density of the unincubated sample; this value times 100 equals the per cent change in pentose.

Menkes (5-8) determined pentose in incubated samples of serum and water and serum and ribose. Colorimetric measurements were made with a Hilger-Spekker colorimeter, and the amount of pentose was calculated from a standard curve. The value of the blank (serum and water) was subtracted from the value of the sample of serum and ribose, and the figure thus obtained was referred to the amount of pentose originally placed in the tube; this gave the per cent of pentolysis. Our method of calculation more accurately estimates change in ribose content, because it is a direct comparison of two identical samples, one incubated and one not incubated.

RESULTS

In Table 1 are shown the results of pentolysis tests performed on eighteen rats with tumors induced by injection of 20-methylcholanthrene. A pentolysis value as great as 10 per cent was not observed in the serum of any of these tumor-bearing rats, and only two sera showed an apparent degradation of pentose greater than 5 per cent. These data, therefore, do not show any evidence of a pentose-destroying factor in the serum of rats bearing tumors produced with 20-methylcholanthrene.

Further studies were made upon rats bearing transplanted tumors with the same incubation procedure and the Roe and Rice (9) and Mejbaum (4) methods for the determination of pentose. Results are shown in Table 2. The decrease in pentose in all instances was less than 5 per cent. These results are further evidence for the non-existence of a pentose-destroying factor in the serum of animals bearing malignant tumors.

DISCUSSION

Since proof of the existence of a pentose-degrading factor in serum is dependent upon the accuracy of the method used for the determination of pentose, three different analytical methods were used.
used, and special attention was directed to a study of the analytical principles involved. Table 3 shows a comparison of the precision obtained by the three methods used. Average variation of the duplicate determinations for both the unincubated and the incubated samples show that the greatest precision in the determination of pentose was obtained by the Roe and Rice method. Results by the Mejbaum method were next in order of precision, and values by the method of Brächet had the least precision. It is to be expected that the method of Brächet for pentose determination, which involves steam distillation, would not give results with the precision obtainable with the Roe and Rice and the Mejbaum methods, which determine pentose in blood filtrates directly. In the reports of Menkes, no mention is made of obtaining large apparent increases of pentose content following incubation. This situation was frequently encountered in our experiments when the Brachet method was employed. In using the method of Brachet, Menkes (5–8) and Steen (10, 11) followed a procedure of outstanding difficulty in which there are considerable possibilities of error.

**SUMMARY**

1. Studies upon the Menkes pentolysis test for malignancy, with three methods for the determination of pentoses in serum, have been made.

2. No evidence was obtained of the presence of a pentose-destroying factor in the sera of rats bearing tumors induced by injection of 20-methylcholanthrene and in the sera of rats with malignant transplanted tumors of four different types.

3. The Roe and Rice procedure for the determination of pentose was found to have greater precision than the methods of Mejbaum and of Brachet.

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**REFERENCES**


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