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Hemolytic Action of Mouse Mammary Carcinoma Filtrate on Mouse Erythrocytes *in vitro*.

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Although the association of cancer with anemia has long been observed, no satisfactory explanation of the mechanism of this relationship has yet been furnished.[1] Experiments reported in this study appear to suggest that, at least in the case of mammary carcinoma of mice, a direct destructive action of a filterable substance contained in the tumor cells, on the erythrocytes of the host, should be taken into consideration. Thus, filtrates prepared from mouse mammary carcinomas were found to destroy mouse erythrocytes *in vitro*. The following technic was employed to demonstrate this hemolytic property of the tumor extracts:

Materials and Methods. Tumor extracts. Ten different mammary carcinomas that developed spontaneously in old C3H females, and 2 transplanted tumors that originated as mammary carcinomas in C3H females and have been carried through successive transplantations in these animals,[2] were used as the source of the tumor extracts. A freshly prepared extract from a single tumor was used for each test. The tumors were removed aseptically, weighed, and ground for several minutes in a porcelain mortar, 0.85% solution of sodium chloride being added to obtain cell suspensions varying from 15 to 30%. The suspensions thus obtained were cleared by centrifugation at 5000 t.p.m. for 10 minutes, and the supernatant fluid was then passed through a Seitz (No. 3) filter; the resulting filtrate was designated "filtered extract," and was used for some of the tests. In other experiments, the tumor cell suspensions were cleared by 3 successive centrifugations at 5000 t.p.m. of 10 minutes each; the final supernatant fluid was designated "centrifugated extract" and used for the tests. Both filtered and centrifugated tumor extracts were bacteriologically sterile as evidenced by negative inoculations of ordinary culture media.

Erythrocytes. Blood was drawn directly from the heart of an etherized mouse of either the C3H[2,3] or Ak[4] inbred lines, or of the An[2] subline. The oxalated red blood corpuscles (RBC) were washed twice at 3,000 t.p.m. for 5 minutes, and a final 1% suspension of RBC in 0.85% solution of sodium chloride was prepared. The RBC suspensions were freshly prepared for each test.

Technic of the test. The entire procedure was aseptic. One cc of a 1% RBC suspension was mixed gently in a small (10 x 75 mm) test tube with 0.5 cc of either centrifugated or filtered tumor extract. Appropriate controls were made simultaneously; they consisted of RBC suspensions to which physiological saline solution, or either filtered or centrifugated extracts from normal, healthy mouse organs (liver, spleen, kidneys) prepared aseptically from mice of the An line known to be free from the mammary carcinoma agent,[2] were added. The test tubes were then placed in an incubator at 37°C for 2½ to 3 hours. After this lapse of time the tubes were removed from the incubator, centrifugated briefly at 3000 t.p.m. and the results were read.

Recording of results. The reading was made in daylight. Complete hemolysis with no sediment left was designated "three plus." Rich in color hemolysis with, however, some sediment left, was designated "two plus." Slight hemolysis was designated "one plus." The faintest trace of hemolysis above the

1 Taylor, A., and Pollack, M. A., *Cancer Research*, 1942, **2**, 223.

2 Gross, L., *J. Immunol.*, 1947, **55**, 297.

3 Strong, L. C., *Genetics*, 1935, **20**, 586.

4 Cole, R. K., and Furth, J., *Cancer Research*, 1941, **1**, 957.

sediment was designated "doubtful." No hemolysis at all, with a colorless supernatant fluid over a sharply demarcated sediment was designated "negative."

Results. Titration of the extracts. Positive results were obtained in practically all tests made with both filtrates and centrifugates obtained from tumor cell suspensions having a concentration varying from 15 to 30%. One plus results were obtained with concentrations varying from 7.5 to 15%. In most instances, either negative, or "doubtful" results could be obtained with 5 or 2.5% concentrations. Higher dilutions gave, as a rule, negative results. There were variations in the intensity of the activity of the various samples of tumor extracts made at different times, and from various tumors. Centrifugates appeared to be only slightly more potent than filtrates. Spontaneous tumors had a stronger hemolytic potency than the transplanted tumors used for the tests.

Under the conditions of our experiments, there were no positive readings among the controls consisting of tubes containing RBC to which physiological saline, or either filtered or centrifugated extracts made from normal healthy organs (liver, kidneys, spleen) removed from An mice[2] were added.

The use of various erythrocytes for the tests. The strongest hemolytic action was observed when filtrates or centrifugates prepared from tumors grown in mice of the C3H line, were mixed with erythrocytes obtained from mice of the same breed. The hemolytic action of these tumor extracts was only slightly weaker, however, for RBC prepared from mice of either the An or Ak inbred lines. In one experiment, erythrocytes were prepared from a "market" albino mouse of unknown parentage, and they were found to be also suitable for the tests. Under the conditions of our experiments, no hemolytic action of either the filtrates or centrifugates was observed when either rabbit, guinea pig, or human erythrocytes were used for the tests.

Exposure of the tumor extracts to 56°C. The hemolytic action of the tumor extracts was found to be only slightly, if at all, inhibited, following exposure of the extracts, prior to tests, to 56°C in a waterbath for 30 minutes. Brief boiling destroyed, however, the hemolytic potency of the tumor extracts.

Hemolytic action of mouse leukemia filtrates on mouse RBC in vitro. Filtrates prepared from either spontaneous, or transplanted lymphatic leukemia of Ak mice[4] were found to possess hemolytic action, similar to that of the mammary carcinoma extracts, *in vitro,* on red blood corpuscles obtained from mice of either the Ak, C3H, or An inbred lines. This action was strongest on erythrocytes prepared from the blood of Ak mice. No hemolytic action occurred when human, rabbit, or guinea pig RBC suspensions were used.

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