TUMOR-HOST RELATIONSHIPS STUDIED IN VITRO: EXPERIMENTS WITH TISSUE SLICES [1]

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Previous studies in our laboratory have shown that the presence of a tumor causes certain chemical changes in the host tissues at a distance from the tumor. These changes are reversed when the tumor regresses (Cerecedo et al., 1959). It seemed of interest to investigate the possibility of duplicating in vitro the results obtained in the intact animal. With this idea in mind, the incorporation of thymidine -H^3 into deoxyribonucleic acid (DNA), and of adenine -8-C^{14} into DNA, and ribonucleic acid (RNA), in slices of tissues of normal and tumor bearing rats has been investigated.

Experimental

Adult male Wistar rats (Carworth Farms, Inc., New City, Rockland

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County, N.Y., or Blue Spruce Farms, Altamount, N.Y.) five to six months old, and bearing the Walker 256 carcinosarcoma; or animals, two to three months old, bearing the Jensen sarcoma, were anesthetized with ether, and killed by exsanguination. Normal rats of similar age were sacrificed as controls. Lungs, liver, spleen, and kidneys were quickly excised and placed in ice-cold physiological saline solution. Tissue slices were made with the aid of the Stadie - Riggs microtome. During the preparation, all tissue was kept between 0° and 4°C. The slices were incubated in the modified Krebs - Ringer medium (Krebs, 1950). When adenine -8-C^{14} (Isotope Specialties Company, Burbank, California) was used as tracer, each flask contained one μc. of the radioactive compound. In the experiments with thymidine-H^3 (Schwarz Bioresearch, Inc., Mt. Vernon, N.Y.) 2.5 μc. of tracer was used. In addition to the thymidine , each flask contained 0.15 μM each of deoxyadenylic, deoxycytidylic, and deoxyguanylic acids. All reaction components were mixed at 0° - 4°C. The incubation took place in 25 ml. Erlenmeyer flasks for 2 hours under oxygen in the Dubnoff shaker at 37 - 38°C. After this time, the reaction was stopped by chilling the flasks to 0°- 4°C. The slices were removed, rinsed several times in ice-cold water, or physiological saline, to remove adhering radioactive material, and frozen on dry ice. At a convenient time, they were thawed and homogenized in cold water. RNA and DNA were isolated by the method of Schneider (1945). Aliquots of the DNA, and the RNA, in trichloroacetic acid (TCA) solution, were plated on stainless steel planchets. The samples were dried, first in a vacuum desiccator overnight, then in the oven at 130°C for one-half hour. They were kept in a desiccator until counted.

Radioactivity was determined in a windowless gas-flow counter. No correction has been made for self-absorption. Since approximately the same

amount of nucleic acid was plated from each solution, the relative activity recorded for each planchet would not be affected by a correction factor. DNA was determined by the method of Dische (1930), RNA by that of Ceriotti (1955).

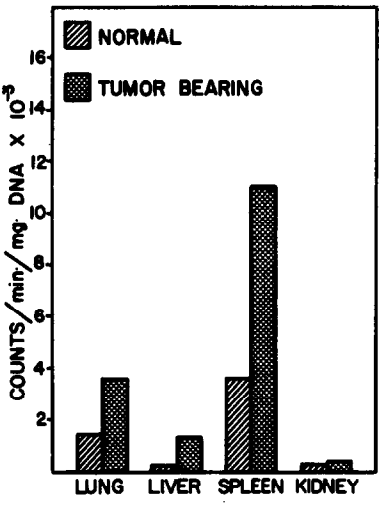

Fig. 1. Incorporation of tritiated thymidine into the DNA of tissues of normal rats and rats bearing the Walker 256 carcinoma.

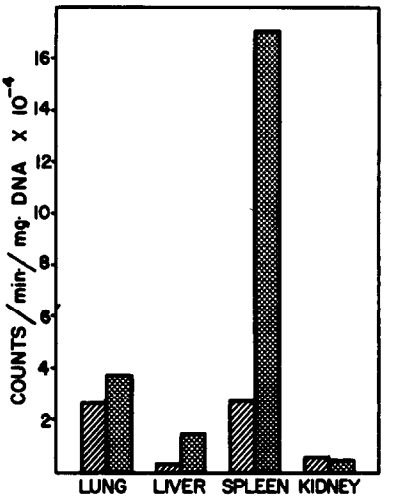

Fig. 2. Incorporation of tritiated thymidine into the DNA of tissues of normal rats and rats bearing the Jensen sarcoma.

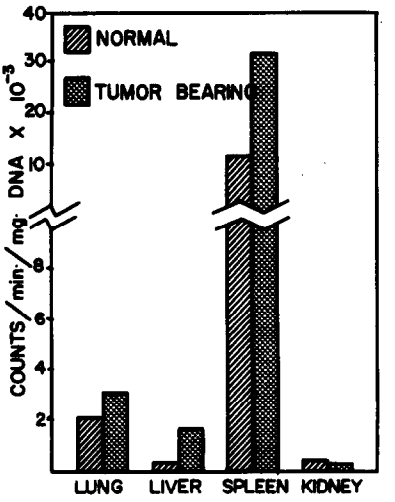

Fig. 3. Incorporation of adenine-8-C^{14} into the DNA of tissues of normal rats and rats bearing the Walker 256 carcinoma.

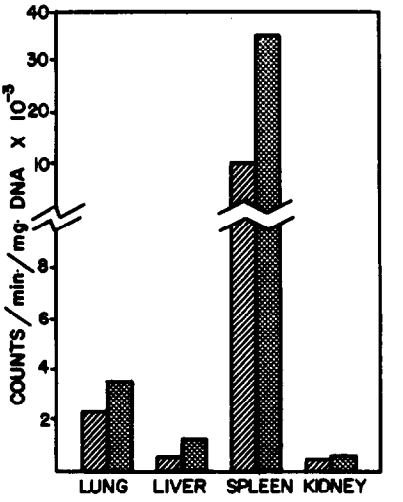

Fig. 4. Incorporation of adenine-8-C^{14} into the DNA of tissues of normal rats and rats bearing the Jensen sarcoma.

Results and Discussion

As shown in Fig. 1, slices of liver and spleen of rats bearing the Walker tumor incorporated thymidine -H^3 into DNA to a markedly greater extent than did the slices of corresponding normal tissues. In the majority of cases, the lung of the tumor - bearing rats showed an increased uptake of the tracer whereas the kidney did not show any change. Similar results were obtained in the tissues of rats bearing the Jensen sarcoma except that the changes observed in the spleen were more pronounced (Fig. 2).

In the experiments with adenine-8-C^{14}, the incorporation of the tracer was determined in the DNA as well as in the RNA. As shown in Fig. 3, in rats bearing the Walker tumor, the DNA in the liver, the spleen, and the lung, showed greater activity while the kidney did not show any change.

The results obtained with the Jensen sarcoma are shown in Fig. 4. The tumor caused an increase in the activity of the DNA of the liver, spleen, and lung. As to the RNA,[4] both tumors caused a slight rise in the activity of the liver. In the other tissues, a drop in the activity was usually observed.

The pattern of thymidine -H^3, and adenine -8-C^{14} incorporation in vitro by the organs of normal and tumor-bearing rats, especially by the liver and spleen, are in harmony with the results which Cerecedo et al. (1959) observed in vivo.

Additional correlation is found in the results obtained with tissues of animals in which the tumor had regressed. Preliminary studies with tissues of such animals show that whatever factor is present in the tissues of tumor-bearing animals which causes an increased uptake of the labeled compound, such factor is absent or becomes much less active after regression of the tumor.

4) Not shown in the Figures.

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Bibliography

1) Cerecedo, L.R., Bresnick, E., and Schubert, E.T. Arch. Biochem. Biophys., 83, 44 (1959).

2) Ceriotti, G., J. Biol. Chem., 214, 59 (1955).

3) Dische, Z., Mikrochemie 8, 4 (1930).

4) Krebs, H.A., Biochim. et Biophys. Acta, 4, 249 (1950).

5) Schneider, W.C., J. Biol. Chem., 161, 293 (1945).