that observed in the fractured right fibula and its normal tibia in the right leg, offers additional proof for our previous reasoning, from the results of breaking strength determinations,[2] that the repair of a fracture is not as local as was formerly thought, and that the skeleton at large is called upon to furnish in a remarkably short space of time those substances essential for the healing of the fracture.

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Viability of the Cells of Rous Chicken Sarcoma Desiccate.

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It is a common experience in preparing tissue cultures, in transplantation and in supravital staining that desiccation is rapidly fatal to mammalian cells. It has usually been assumed that the tissues of all higher animals are similarly injured. Nakahara,[1] however, found in the desiccate of the Rous chicken sarcoma cells that he thought to be alive. He treated the cells of a desiccate more than 4 months old with dilute solutions of trypan blue and also inoculated fresh chicken plasma with them. By both methods cells thought to be living were seen.

Since Nakahara apparently observed the cultures for only a short time (3 or 4 hours) it seemed desirable to keep cultures under observation for a longer period. A preparation of desiccate was generously furnished me by Dr. Rous. It was prepared November 16, 1925, and on June 2, 1926, several dozen cultures were made employing the usual cover-glass method. The desiccate was first rubbed up with a small amount of Ringer solution to make a thick paste which was kept on ice until the explants were made. In one series whole fresh Barred Rock plasma was inoculated with the paste and in another the plasma was diluted with one-third volume of distilled water. The cultures were at once placed in the incubator and examined at 3 and 5 hours, one, two and five days. Round cells of medium size were seen on first examination and suggested growth. The nuclei were perfectly distinct and their irregular outline might be interpreted as evidence of ameboid motion. Many of these cells were located by ink marks on the cover-glass. After 5

[1] Nakahara, W., *Gann. Japanese J. Cancer Res.*, 1926, **20**, 13.

days there was no change in position or outline of these cells. In places at the periphery of the explant spindle cells projected outward radially into the plasma. These likewise remained stationary during the 5 days of incubation. On June 2, three hens were inoculated with the same paste from which the cultures were made. The hens were mongrels having atypical Barred Rock markings. Two of them developed large tumors the latter part of July. July 30 a desiccate was made from one of these by drying over phosphorus pentoxide in partial vacuum at a temperature below 32°C. On September 21, 1926, when completely dry this was sealed in small tubes, In early June, 1927, several baby chicks were inoculated in the pectoral muscle and those that lived one month developed tumors. At this time explants to heparinized chicken plasma were made and clotting produced with embryo extract. The results were the same as those obtained with the original desiccate.

The fresh living cells of the Rous sarcoma both round and spindle-shaped when examined directly with dilute neutral red react with the appearance of characteristic cytoplasmic granules.[2] Many examinations of the desiccate were made by this method. Cytoplasmic granulation appeared in none of the cells. Although there were differences in the depth of staining both nuclei and cytoplasm tended to stain faintly and diffusely.

Conclusions. In 2 desiccated preparations of Rous chicken sarcoma viable cells were not demonstrable by the tissue culture method. By the method of supravital staining with neutral red the reaction of the desiccate was unlike that given by the living sarcoma cells.

This work was begun in the Department of Pathology, Washington University, St. Louis, Missouri, and I am indebted to Professor Leo Loeb for the privilege of publishing those results along with the ones obtained here.

[2] McJunkin, F. A., *J. Cancer Res.*, 1926, **12**, 47.