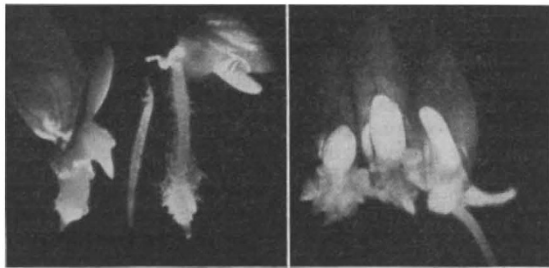


pulp, covered with filter paper in Petri dishes. The seedlings that developed at a distance of 1.5–2.5 cm. from the pulp in the dishes showed similar symptoms. Morphologically abnormal seedlings had cells and group of cells with altered chromosome sets (chiefly polyploid) like the seedlings treated with colchicine<sup>9</sup>. Seeds germinating on disintegrating pieces of bulb from *Colchicum* sp. or near them showed the same morphological abnormalities, namely swelling of the roots and the stems. They also had polyploid cells and tissues. Morphological abnormalities and chromosome alterations (chiefly polyploidy) were also induced when seeds were grown in *Colchicum* pulp



(a) IN THE MIDDLE—A NORMAL (CONTROL) ROOT FROM A WHEAT SEEDLING; ON EACH SIDE, ROOTS FROM SEEDLINGS GROWN (LEFT) ON *Colchicum* PULP AND (RIGHT) AT A DISTANCE OF ABOUT 2 CM. FROM THE PULP IN PETRI DISH WITH FILTER PAPER. (b) RIGHT, A NORMAL WHEAT SEEDLING; THE OTHER TWO, GROWN ON PULP FROM *Colchicum*.

mixed with sand in pots or on disintegrating pieces of various size from *Colchicum* in pots with soil and sand. These observations show that: (1) Pulp from *Colchicum* bulbs can be used for experimental production of polyploid plants. (2) Disintegrating bulbs from *Colchicum* inducing chromosome alterations in laboratory conditions should also cause the same phenomenon in Nature.

The foregoing investigations suggest searching for polyploid forms among the plant species living in Nature together with *Colchicum*.

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<sup>1</sup> Extensive literature list, see in Kostoff and Sarana, *J. Genetics* (in the Press).

<sup>2</sup> Kostoff and Kendall, *Biol. Bull.*, 56, 402–459 (1929).

<sup>3</sup> Kostoff and Kendall, *Zentralbl. f. Bakter.*, II Abt., 81, 86–90 (1930).

<sup>4</sup> Kostoff and Kendall, *J. Genetics*, 21, 113–115 (1929).

<sup>5</sup> Kostoff and Kendall, *Genetica*, 12, 140–144 (1930).

<sup>6</sup> Kostoff and Kendall, *Science*, 76, 144 (1932); *Archiv. f. Mikrobiol.*, 4, 487–508 (1933); *Gartenbauwissenschaft*, 9, 20–44 (1934).

<sup>7</sup> Kostoff, *Ann. Agr. Fak. Sofia Univ.*, 8, 317–324 (1929–30).

<sup>8</sup> Kostoff, *Genetica*, 15, 103–114 (1933); *Phytopathol. Z.*, 9, 387–405 (1936); *Current Sci.*, 6, 555–556 (1938).

<sup>9</sup> Kostoff, *NATURE*, 142, 753 (1938).

### Transformation *in vitro* of Cultures of Normal Cells Treated with Rous Sarcoma Agent into Sarcoma Cultures

THE procedure adopted for the cultivation of the Rous sarcoma *in vitro* in accordance with the usual method described by A. Fischer<sup>1</sup> is as follows: A fragment of tumour is incubated in a medium consisting of chicken plasma and dilute embryonic extract. After a time the growing culture begins to digest the surrounding medium and also itself. Such

a culture will die out if it is not provided with fresh tissue by means of subculture. The sarcoma cells infiltrate the added tissue fragment within which they remain for a time embedded. If normal tissue is regularly added, the sarcoma cells can be cultured over an unlimited period and will retain their malignancy.

Since A. Fischer succeeded in culturing sarcoma tissue by the addition of dead tissue (kept in 'Tyrode' in the refrigerator for a month), it might be assumed that the added tissue has a mechanical and perhaps nutritive function in the cultivation of sarcoma, and that the growth of sarcoma *in vitro* depends solely on the continuous multiplication of the original malignant cells. On the other hand, the early experiments of A. Carrel<sup>2</sup> and those recently published by Ludford<sup>3</sup> show that the Rous sarcoma agent as such is capable of 'infecting' normal cells *in vitro*. We therefore felt justified in assuming that in *in vitro* cultivation of sarcoma according to the methods described above, some process may be taking place in addition to the multiplication of the original sarcoma cells. It may be assumed that the normal cell material which is added is influenced by the agent present in the original sarcoma culture and as a result there is, in addition to a multiplication of sarcoma cells, a constant process of 'cancerization' of the newly added normal tissue.

The first essential in the clearing up of this problem experimentally is the definite separation in culture of agent from the multiplying sarcoma cells. We have succeeded in separating agent from living cells *in vitro* by irradiating cultures with radium.

The relationship between the dose needed to kill cells and that needed to destroy the agent in Rous sarcoma cultures was established accurately. It was, therefore, possible to kill the cells without appreciably impairing the activity of the agent.

An eight-year-old Rous culture strain given us by A. Fischer was used for the experiments. A radium plate containing 6 mgm. of radium was employed, the  $\beta$ -rays being used<sup>4,5</sup>.

The experiments have shown that the addition of a fragment of tissue or of a pure culture of normal fibroblasts to an irradiated culture of Rous sarcoma, which had received double the dose needed to kill the cells (under the conditions of our experiments this involves an irradiation time of seventy-two hours), results in an invasion of the normal cells by the still active agent. On subculture in a hanging-drop, such a cell colony carrying agent acquires all the known morphological and physiological properties of a real Rous sarcoma culture (typical cell forms, autolytic power, typical behaviour with added cell material). Inoculation into a fowl always proves such a culture to be malignant, causing a typical sarcoma. The use of strongly irradiated cultures causes the 'cancerizing' process to last longer, and the transformation of normal cell colonies in Rous sarcoma cultures manifests itself only after 6–8 passages.

Details of our work will shortly appear elsewhere.

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<sup>1</sup> "Gewebezüchtung", Munich (1930).

<sup>2</sup> *J. Exp. Med.*, 43, 647 (1926).

<sup>3</sup> *Amer. J. Canc.*, 31, 414 (1937).

<sup>4</sup> *NATURE*, 139, 841 (1937).

<sup>5</sup> *Arch. exp. Zellforsch.*, 19, 475 (1937).