

squash seedlings. They find that increased nitrogen results in increased shoot development and increased carbohydrates stimulate root development. Hicks⁵ has applied this relationship to explain polarity in regeneration in the willow (*Salix viminalis*). She states that prior to development, nitrogen is translocated to the apex and carbon flows to the base. Shoots develop in the area (apex) of lowest C/N ratio, and roots appear in the area (base) of highest C/N ratio. The writer, working with *Salix nigra*, found that Hicks's results hold for cuttings having a normal gradient of total nitrogen (from morphological apex to the morphological base), in a normal position, and with a well-developed shoot and root polarity. Under such conditions, shoots develop in the area (apex) of highest total nitrogen per gram dry weight of cutting, and roots appear in the area (base) of lowest total nitrogen per gram dry weight of the cutting. Inverted cuttings, however, show different results. *A*, Fig. 1, shows an inverted cutting

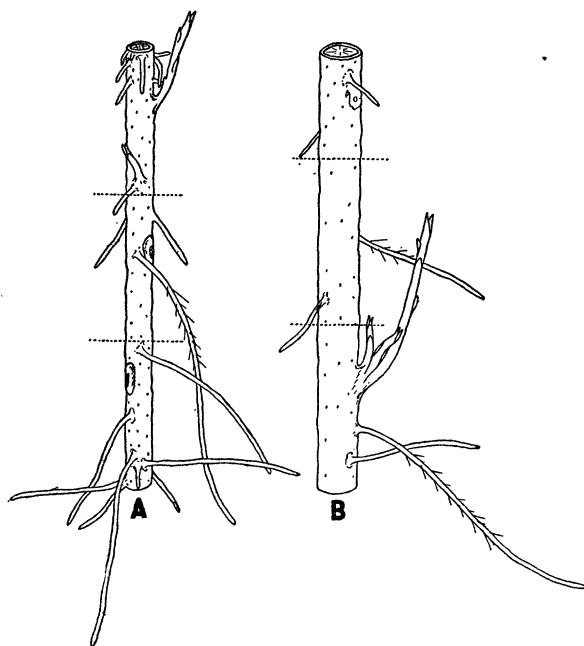


FIG. 1. *A*, an inverted cutting in which the gradient of total nitrogen is from the morphological base to the apex. *B*, an inverted cutting in which the gradient of total nitrogen is from the morphological apex to the base. The parts above and below the dotted lines were used for total nitrogen analyses.

in which the gradient of total nitrogen was from the morphological base to the apex, rather than from the morphological apex to the base, as is found in normal cuttings. The total nitrogen analyses (Table I) show that shoots develop in the area (morphological base)

⁵ *Bot. Gaz.*, 86: 193-209, 1928.

TABLE I

TOTAL NITROGEN ANALYSES OF CUTTING A

The total nitrogen of the bark and wood is shown in milligrams of nitrogen per gram dry weight of the material. The total nitrogen of the roots and shoots was adjusted to the dry weight of the bark.

Part used for nitrogen determination	mgs N per gm dry weight			
	bark	shoots	roots	wood
Morphological apex	17.3259		0.1359	2.3316
Morphological base	16.9161	1.0304	0.0453	2.0299

of highest total nitrogen per gram dry weight of the cutting, while roots regenerate along the entire cutting and are not confined to the area (morphological apex) of lowest total nitrogen per gram dry weight of cutting. *B*, Fig. 1, shows an inverted cutting with a normal gradient of total nitrogen from the morphological apex to the base. Total nitrogen analyses (Table II) show that shoots develop in the area (mor-

TABLE II

TOTAL NITROGEN ANALYSES OF CUTTING B

The total nitrogen of the bark and wood is shown in milligrams of nitrogen per gram dry weight of the material. The total nitrogen of the roots and shoots was adjusted to the dry weight of the bark.

Part used for nitrogen determination	mgs N per gm dry weight			
	bark	shoots	roots	wood
Morphological apex	15.3337	1.1389	0.0765	2.3609
Morphological base	15.4889		0.0201	2.6144

phological apex) of highest total nitrogen per gram dry weight of the cutting and roots appear (as in cutting *A*) along the entire cutting.

The data indicate that regardless of the position of the nitrogen gradient and the position of the cutting, shoots develop in the area of highest total nitrogen per gram dry weight, while roots are not confined solely to the area of lowest total nitrogen per gram dry weight, but may appear along the entire cutting.

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EFFECT OF FREEZING AND THAWING ON THE EXTRACTABILITY OF THE AL- LEGED CHICKEN SARCOMA AGENT¹

THE assumed cell-free transmission of chicken sarcomas has given rise to many speculations as to the possible nature of the transmitting agent. We purposely avoid discussing relative merits of various

¹ Based on a paper read before the National Medical Congress of Japan, Osaka, April 4, 1930.

notions that have been suggested, but refer only to the one recently advocated by certain authorities, namely, that the so-called chicken sarcoma agent may be an enzyme-like substance.

The well-known fact that the "agent" is completely destroyed by an exposure to the temperature of 55° C. for 15 minutes (Rous) seems to have been entirely forgotten by the advocates of the "enzyme theory." In addition we previously showed that a potent desiccate of the sarcoma tissue can be inactivated mechanically by grinding it up in a mortar into extremely fine powder, indicating that the transmitting agent may be a formed body, not a chemical substance. In this paper we report another evidence, which may well be final. It is based on the freezing and thawing method of extracting endocellular enzymes.

Mashed Rous sarcoma tissue was divided into two portions, one of which was subjected to the process of repeated freezing and thawing. The freezing was done in a test-tube by means of the freezing mixture of ice and salt (temperature of -18° C. for 20 minutes) or with the aid of dry ice (-75° C. for 10 minutes), and thawing was accomplished by dipping the test-tube in water bath at the temperature of 37° C. for from 5 to 10 minutes. After repeating this process of freezing and thawing seven to ten times, the sarcoma material was extracted with 20 times its volume of physiological saline solution for 10 minutes with constant shaking, and then centrifuged. Simultaneously the control portion, which had remained in the ice-box, was similarly extracted and centrifuged under identical conditions.

The supernatant fluid (extract) of the two materials were then separately inoculated into normal chickens, the treated extract into the left, and the same amount of the control extract into the right pectoral muscle. It was noted that extracts from the treated material contained a far smaller number of sarcoma cells than the control extracts. Sediments of the treated and control materials were also similarly tested as to their comparative tumor-producing actions.

The result of such inoculations into 30 chickens demonstrated conclusively that the process of repeated freezing and thawing very strikingly reduces the tumor-transmitting action of the sarcoma materials, both extract and sediment. With untreated materials, large tumors, often replacing the entire "breast," were produced in 7 to 10 days, while tumors resulting from treated materials were always decidedly smaller. Moreover, treated materials failed to produce tumors in 5 out of 30 cases, but untreated materials gave rise to a tumor in every case.

The point which we consider most significant in these results is the very feeble tumor-producing action of the extract from the sarcoma tissue subjected to repeated freezing and thawing. This process disrupts many sarcoma cells and should facilitate the liberation of any enzyme-like substance contained in the cells. Therefore, if an enzyme-like tumor-producing agent were contained in the sarcoma cells, extracts obtained from disrupted cells should be much more potent than extracts obtained from untreated, intact cells. Our results radically contradict this expectation and show that the extracts obtained from disrupted sarcoma cells are much less active than those prepared from intact cells. On the basis of this fact we conclude that the Rous chicken sarcoma does not contain any enzyme-like agent capable of sarcoma transmission.

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