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 inactivat-
ed mechanically by grinding it up in a mortar
into extremely fine powder, indicating that the trans-
mittng 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 min-
utes) 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 centri-
fuged. 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 ma-
terials 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 simi-
larly tested as to their comparative tumor-producing
actions.

The result of such inoculations into 30 chickens
demonstrated conclusively that the process of re-
peted freezing and thawing very strikingly reduces
the tumor-transmitting action of the sarcoma ma-
terials, 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
decisively 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 dis-
rupts many sarcoma cells and should facilitate the
liberation of any enzyme-like substance contained in
the cells. Therefore, if an enzyme-like tumor-pro-
ducing agent were contained in the sarcoma cells,
extracts obtained from disrupted cells should be much
more potent than extracts obtained from untreated,
tumor 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 sar-
coma does not contain any enzyme-like agent capable
of sarcoma transmission.

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