A STUDY OF SERUM OF CHICKENS RESISTANT TO ROUS SARCOMA.

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The purpose of this paper is to report some experiments carried out with serum of chickens which had been found to be resistant to Rous sarcoma. A study of these resistant birds has been reported in previous communications. A resistant bird is one in which a well developed tumor regresses; on further transplantation small nodules may develop, but these also regress, and the bird does not die of tumor.

Pure-bred barred Plymouth Rock chickens obtained from the Ontario Agricultural College were used exclusively. The birds were from three to twelve months old. Tumor extract was prepared as follows: 3.0 gm. of tumor tissue were ground with sand and 3 c.c. of concentrated saline; this was allowed to stand fifteen minutes; 100 c.c. of distilled water were then added and the mixture was centrifuged. In some cases this extract was filtered through layers of sand and paper pulp, as described by Gye. This type of extract was called sand filtrate. Only one injection was given to each bird at one time. As a rule, three birds were used to test each serum mixture. All tumor extracts or sand filtrates were tested on three birds. Sodium citrate was used when drawing blood to prevent clotting. In some instances heparin was also used.

The first investigation with resistant serum was to ascertain if it neutralized active tumor extracts. On June 23, 1930, the serum of resistant bird No. 149 was tested. Three tubes were prepared as follows: (1) 9 c.c. of resistant chicken serum + 3 c.c. of tumor extract; (2) 9 c.c. of normal chicken serum + 3 c.c. of tumor extract; (3) 9 c.c. of normal saline + 3 c.c. of tumor extract. All tubes were incubated for half an hour in a water bath at 37.5° C. Nine birds were used, three for each mixture.

The birds which received the saline-tumor extract mixture died in an average of thirty-five days. The birds which received the normal serum-tumor extract mixture developed tumors and died in an average of forty-one days. In the three birds which received the mixture of resistant serum and tumor extract no tumor developed. One of these birds died of other causes in fifty-three days; the other two were still negative in 175 days, at which time they were given a direct transplant and died of tumor in eighteen and twenty-two days.

This experiment was repeated under similar conditions six weeks later (August 1, 1930). The saline-tumor extract control birds died

\footnote{Read before the American Association for Cancer Research, Toronto, March 28, 1934. For discussion, see page 670.
in thirty-two, twenty-seven, and forty-five days; the normal serum-tumor extract birds died of tumor in forty-one, twenty-five, and thirty-four days. One of the resistant serum-tumor extract birds did not develop a tumor; the other two died of tumor in forty-five and seventy-five days respectively.

From Feb. 19, 1930, to Jan. 18, 1934, the neutralizing power of the serum of resistant birds was tested forty times. The amount of serum used to neutralize 1 c.c. of tumor extract varied from 0.1 c.c. to 3 c.c. The time of incubation varied from thirty minutes to three and a half hours. In 14 of the 40 tests the tumor extract was completely neutralized, as indicated by the fact that all three birds remained negative. In 13 tests, two birds remained negative and one became positive in each group. In 11 tests, one bird remained negative and 2 became positive, and in 2 tests, the first two of the series, all birds became positive and died of tumor.

From these experiments it is obvious that resistant serum did not always completely neutralize a tumor extract. This was partially explained by the variation in the amount of serum used and the time of incubation. However, in interpreting these results three additional factors must be borne in mind: (1) the neutralizing activity of a resistant serum may vary from time to time; (2) the potency of the tumor extracts used cannot be predicted; (3) the response of the birds receiving the mixture is not constant. When tumors do occur from a resistant serum-tumor extract mixture, they are usually delayed in appearing and are hard and slow-growing.

In order to ascertain the influence of incubation on the neutralizing effect of the resistant serum, experiments were performed in which the serum and tumor extract were mixed and given immediately. In every experiment one or two of the three birds failed to develop a tumor. However, it was still doubtful as to whether the neutralization took place in the body or in the test tube. In order to settle this point another experiment was planned:

Nine birds were given 1 c.c. each of turpentine, diluted 1:40, in the right breast. The following day three of the birds were given intravenously 10 c.c. of a mixture consisting of 15 c.c. of resistant serum and 15 c.c. of a potent sand filtrate, which had been incubated at 37.5° C. for three hours. Three of the birds received intravenously 10 c.c. of a mixture consisting of 15 c.c. of normal chicken serum and 15 c.c. of the same sand filtrate, incubated for three hours. The other three birds received 5 c.c. of resistant serum and 5 c.c. of the same sand filtrate, given through the same needle, from different syringes, and washed in with 2 c.c. normal saline. The three birds which received the incubated normal serum-sand filtrate mixture all developed a tumor at the inflamed area of the turpentine injection and died of tumor in forty-five, twenty-seven, and twenty-eight days. The birds which received the non-incubated resistant serum and sand filtrate mixture all developed breast tumors and died in thirty-eight, thirty-nine, and forty-six days. The birds which received the incubated resistant serum-
sand filtrate mixture did not develop breast tumors. In one of these birds, unfortunately, a tumor developed in the wing, caused by a small amount of the mixture going under the skin at the time of inoculation. This experiment indicates that the neutralizing effect of the resistant serum occurred in the test tube during incubation.

Resistant serum was tested in a number of experiments to ascertain at what temperature its neutralizing power was destroyed. In these experiments 3 c.c. of serum were diluted to 9 c.c. with normal saline. The tubes were heated and the temperature of the serum was maintained at 60, 70, 80 and 90° C. for five minutes. After cooling, 3 c.c. of tumor extract were added to each tube, and the mixtures were incubated at 37.5° C. for an hour and a half. Birds which received the mixtures heated to 60° and 70° did not develop tumors. Birds which received the mixtures heated to between 80° and 90° all developed tumors. This indicates that the neutralizing effect of the resistant serum was destroyed between 70 and 80° C.

A number of experiments were carried out with the fractions of resistant serum obtained by one-third, one-half, and full saturation with ammonium sulphate. At this time the amount of resistant serum was limited, but it appeared that the neutralizing fraction was precipitated at one-third saturation.

Although serum of a bird resistant to Rous sarcoma neutralizes tumor extracts, the effect of administering it intravenously to tumor-bearing birds is disappointing. On Oct. 31, 1930, five birds were given a direct transplant and four days later two of the birds were given 7 c.c. of resistant serum intravenously. From 6 to 10 c.c. of resistant serum were given daily for eleven days. One bird received 72 c.c. and died in nineteen days with a large tumor and secondaries in the liver. The other bird received 90 c.c. of serum, survived thirty-two days, and died with a large breast tumor and secondaries in the heart and lungs. The three control birds died of tumor in twenty-six, thirty-three, and twenty-five days.

This experiment was repeated when we had fifteen resistant birds, thus enabling us to use larger doses of serum. Blood was drawn from each of the fifteen resistant birds once a week and the mixed serum was used for treatment. Incubation tests showed that 0.1 c.c. of this mixed resistant serum completely neutralized 1 c.c. of a potent tumor extract. A bird received 1 c.c. of sand filtrate and eleven days later, when the tumor measured 2 × 2.2 cm., resistant serum treatment was begun. The bird was given 15 c.c. of serum twice a day for the first week and 10 c.c. daily for the second week. In spite of the serum treatment, the tumor grew rapidly and the bird died in thirty days with a large breast tumor and secondaries in the lungs. Untreated birds receiving the same sand filtrate died in twenty-five and thirty-nine days. The treated bird received 283 c.c. of resistant serum without any apparent beneficial effect.

On Dec. 29, 1933, two of our Rous resistant birds, Nos. 373 and 1038, were given an extract of dried Fujinami powder. Chicken No.
373 had been under observation for three years, during which time it remained resistant to seventeen direct transplants of Rous sarcoma. Chicken No. 1038 had been under observation for over two years and had remained resistant to eleven Rous transplants. Both these birds developed large tumors following the Fujinami inoculation and died with secondaries in the lungs in thirty-five and forty-nine days respectively. Blood was obtained from the heart of bird 373 at autopsy and the serum was found to neutralize Rous sand filtrate completely. Serum of bird 1038, obtained within twenty-four hours of death, also neutralized Rous extract. This would indicate that these birds remained resistant to Rous sarcoma, although dying of Fujinami tumor.

Having obtained a barred Plymouth Rock chicken resistant to Fujinami tumor, it was decided to test its serum. It was found that resistant Fujinami serum neutralized a potent Fujinami extract but had no effect on a Rous sand filtrate. Conversely, resistant Rous serum neutralized Rous extracts but had no effect on Fujinami extracts. The Fujinami resistant bird was transplanted subsequently with Rous cells and developed a tumor.

Conclusions

1. Rous resistant serum neutralizes Rous tumor extract; this neutralization occurs during incubation.
2. The neutralizing power of Rous resistant serum is destroyed between 70 and 80° C.
3. Intravenous administration of Rous resistant serum has no effect on a growing Rous tumor.
4. Fujinami resistant serum does not neutralize Rous tumor extract.
5. Rous resistant serum does not neutralize Fujinami extract.