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Observations on Gye's work with the Rous sarcoma.

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The writer wishes to report briefly the results of experiments carried out during the last seven months in attempting to repeat and amplify the experiments of Gye[1] on the Rous chicken sarcoma. The latter interpreted his results as meaning that two factors were involved in the production of this tumor; first a specific, non-living, chemical factor found only in extracts of the neoplasm; and second, a filtering virus, capable of being cultivated *in vitro* under certain conditions. The latter was also obtainable from cultures of mammalian tumors. Neither substance alone was capable of producing a tumor, together they were active. The media used in cultivation of the virus contained broth, rabbit serum and chick embryonic tissue. No controls using uninoculated, but incubated media of this type to replace virus were quoted in the original paper. In personal communications, Gye has reported to the writer that such controls have been carried out and in his hands have proved negative. Murphy,[2] on the contrary, has recently presented evidence that media uninoculated with tumor, but containing either chick embryonic tissue or mouse placenta, i. e., rapidly growing tissues, and incubated anaerobically, could be substituted successfully for virus cultures. Until agreement can be reached on this and other points, judgment of the interpretations to be placed on the phenomenon may well be reserved.

In this laboratory the repetition of the type experiments of Gye have proved to be far from easy. The experiments thus far have used something over two hundred chickens and have served to point out the difficulties to be expected, and perhaps to suggest means of overcoming them.

There are marked individual variations in susceptibility to

[1] Gye, W. E., *Lancet*, 1925, ccix, 109.

[2] Murphy, James B., *J. Am. Med. Assn.*, 1926, lxxxvi, 1270.

small doses of tumor extract manifested even by pure Barred Rock chickens, which we have used exclusively. This may apparently be overcome to a large extent by the use of fairly young fowls of approximately the same age. We are at present using chicks of six to eight weeks of age, hatched from eggs coming as far as possible from the same flock of hens, incubated and raised in the laboratory. Much importance may rest on whether tests and controls are carried out on the same or different chickens. Criticisms of either method may be advanced.

Most important of all the technical points is undoubtedly the preparation of the specific factor by the destruction of virus in the tumor filtrates by means of chloroform. (The terminology of Gye's conclusions is here used for the sake of simplicity.) This was emphasized by Gye in his publication, but an additional difficulty is introduced by the fact, not mentioned by him, and perhaps not true of the tumor in his hands, that the susceptibility to chloroform of the virus obtained from tumors in different chickens appears to vary tremendously. In the experiments summarized here, roughly half of the filtrates treated with chloroform have failed to produce tumors either alone or when mixed with virus cultures. In the other half, tumors have been produced by both the mixture and the control. In other words, about half of the chloroform filtrates have been too severely inactivated by the reagent, and in the case of the others the treatment has been too mild. Obviously, no standardization involving quantity of chloroform, time of treatment and method of mixing will be of any avail as long as individual tumors show marked differences in their response. At present the writer is attempting to overcome this difficulty by departing rather widely from Gye's actual technique by the use of dessicated tumor in place of fresh tumor in the preparation of filtrates. As Rous, Murphy and Tytler[3] showed in their early work on this tumor, drying does not destroy the infectious agent. By preparing a sufficient quantity of dessicated tumor to carry out a number of experiments it should be possible to so standardize the method as to give uniform filtrates which can be brought with some certainty by means of chloroform to a point where it will just fail to infect most chickens in given

[3] Rous, P., Murphy, J. B., and Tytler, W. H., *J. Am. Med. Assn.*, 1912, lviii, 1682.

dosage. Results up to the present are encouraging. Filtrates prepared from dessicated material are infectious in approximately the same quantities as those prepared from corresponding amounts of fresh tumor tissue. They are apparently rendered inactive rather more easily by chloroform than the latter. It is too early yet to say how uniform the resistance to chloroform will prove to be. Moreover, in two chickens out of four in one group injected in one breast with a mixture of a chloroform filtrate and a virus, and in the other with the chloroform filtrate alone, tumors have developed from the latter injection, and not from the former.

It seems that the greatest hope of clearing up the question involved in this work must come through a method which will give predictable results in controls and type tests in all, or nearly all chickens inoculated. We have had a few experiments, not more than two or three, in which it would be possible to pick out from a dozen chickens inoculated three or four in which a perfect type experiment was shown. In one experiment in particular, owing to a peculiar and entirely accidental grouping of controls and tests both on the same and on different chickens, it is not unlikely that positive results were really obtained, although certain of the controls were also positive. There is apparently reason to believe that by means of a uniform dessicated preparation, similar results may be obtained in the majority of experiments, and it will then be possible through an extension of controls to arrive at a more exact understanding of the reasons underlying Gye's experiments.

To summarize, in order to repeat and more adequately control Gye's experiments, it is essential to standardize every variable factor as far as possible. We believe the differing susceptibility on the part of the chickens may be largely overcome by using young chickens of about the same age from the same blood related flock. The most important variable is the resistance to chloroform of individual tumors. It appears that this may be largely obviated by substituting dessicated material made in considerable quantity for fresh tumor in the preparation of the specific factor. While we have obtained occasional indications of successful experiments of Gye's type, little can be learned from them unless they can be produced with considerable uniformity.

Many of these experiments have been carried out with the assistance of Miss Alberta Marx, and Mr. Ashton Graybiel, to whom I wish to express my appreciation and thanks.

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Extent of capillary bed and rôle of Thebesian vessels in coronary circulation.

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Intracardiac injections of dyes and of India ink in living cats, rabbits and rats have resulted in complete filling of the capillaries of these hearts. Numerous counts have shown approximately 1100 capillaries to each thousand muscle fibers, or about one capillary to each muscle fiber. But when for any reason the heart dilated during the injection, very few of the capillaries were injected, though the larger vessels were completely injected.

The same results were obtained when human hearts were injected through the coronary arteries. Distension of the chambers prevented injection of the capillaries, but when steps were taken to prevent dilatation of the chambers complete injections were obtained.

At the same time it was noted that perfusion of the coronary arteries in dead hearts resulted in distension of the chambers, and when the walls were so stretched 80 per cent to 90 per cent of the perfusate escaped directly into the chambers of the heart, while only 10 per cent to 20 per cent returned by way of the coronary sinus and veins. These findings suggest that during dilatation of the heart the chief route of blood flow is through the arteries to the Thebesian vessels and thence into the chambers of the heart.

A modification of the Langendorff method of coronary perfusion has given complete injections of the capillaries in cat and rabbit hearts when the hearts were beating strongly. This meth-