A CHANGE IN RABBIT FIBROMA VIRUS SUGGESTING MUTATION

II. Behavior of the Variant Virus in Cottontail Rabbits

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Andrewes (1) has observed a change in the type of lesion produced by a strain of the rabbit fibroma virus. Instead of causing fibroma-like tumors at sites of inoculation as at first (2) it produced in his experience only acute inflammatory lesions. The characteristics of this "inflammatory" type of virus have been described in an accompanying paper (1). A similar but less complete change in the virus has been noted in this laboratory. It is the purpose of the present paper to describe the change briefly and to outline experiments in which attempts were made to cause the virus to revert to the original type.

A Change in the Type of Lesion Produced by the Fibroma Virus

The virus used was the one studied in the original experiments (2) and designated, for convenience, as Strain A to distinguish it from viruses since obtained from fibromata found on 4 other cottontail rabbits. Originally and for a number of serial passages in domestic rabbits this virus had produced, when injected intratesticularly, a marked proliferation of connective tissue throughout the testicle and epididymis. When administered subcutaneously it produced at the site of inoculation a large swelling which had the gross and microscopic appearance of a fibroma. During the early intensive work with the virus it was passed at frequent intervals with at most short periods of storage in glycerol, while later it was transferred less frequently and after longer storage in glycerol. At each serial passage the virus was inoculated both subcutaneously and intratesticularly
into domestic rabbits, but usually only the fibromatous testicles were saved as a source of virus for infecting rabbits of the subsequent passage. Fibromatous infiltration of the testicles and subcutaneous tissue at the point of inoculation was noted at each passage. If the virus used in a given passage had been stored unusually long in glycerol the subcutaneous fibroma induced was in some instances quite small. This was thought to be due to loss or inactivation of part of the virus by prolonged storage. Direct passage from such a fibroma again yielded a typical large growth on subcutaneous inoculation.

No growth resulted at the site of subcutaneous injection in the rabbit of the 18th serial passage, but the 19th animal, injected with testicle from the 18th, developed a fairly large subcutaneous fibroma. Since then, in 12 further passages, the virus has failed to induce subcutaneous fibromata apparent in the gross. Andrewes had been supplied with virus from the 18th passage rabbit. It was thus evident that, at approximately the time that Andrewes noted that the fibroma virus no longer produced fibromatous lesions, a change had also occurred in the virus in this laboratory.

In addition to losing its ability to produce subcutaneous fibromata, my virus no longer caused the same extensive fibromatous proliferation in the testicles as formerly. The inoculated testicles, however, became firm, were swollen and inflamed, and scrotal edema was encountered quite frequently. Histologically, the picture seen in the testicle was now quite different from that originally produced by the virus. Instead of an extensive infiltration with young connective tissue cells, the spaces about the seminiferous tubules were densely packed with lymphocytes and large mononuclear cells. There was an accompanying atrophy of the tubule cells. As a rule only small and isolated islands of fibroblasts were to be seen, but in some passages the reaction was predominantly fibromatous. The subcutaneous tissue when inoculated with the virus also showed accumulations of lymphocytes and large mononuclear cells. The fibroblast-like cells so prominent formerly were now almost completely lacking. Virus administered intracutaneously produced raised, reddened, glistening papules which were similar histologically to the subcutaneous lesions. The change noted in the virus in this laboratory was thus apparently of the same general nature as that observed by Andrewes. It differed, however, in degree, for while Andrewes' virus produced only an inflammatory reaction when injected intratesticularly, the 'changed virus' of this laboratory produced, in addition, a varying but usually scant connective tissue proliferation. Small satellite nodules were observed occasionally close to the inflammatory reaction at the site of subcutaneous injection or about the papules.
arising where the virus was inoculated into the skin. The pock-like lesions described by Andrewes have not, however, been encountered.

Experiments with the Original Fibroma Virus

After several rapid serial transfers of the virus beyond the 19th passage had failed to restore its ability to produce fibromata when administered subcutaneously, the question arose as to whether the apparent change in the type of lesion produced was due to an alteration in the virus or to some variation in the host. So far as could be observed, the rabbits, now developing but scanty inflammatory reactions following subcutaneous inoculation with the virus, were similar in all respects to those earlier ones which had developed large subcutaneous fibromata. In order to settle the question, a second series of experiments was begun. The original cottontail rabbit fibroma which had furnished the virus under study had been in glycerol in the refrigerator for 18 months by now. A portion was ground, suspended in saline in the usual way, and used to inoculate a rabbit subcutaneously and intratesticularly. After an unusually long incubation period fibromatous nodules developed in the testicles. Passage from these yielded typical subcutaneous and testicular fibromata similar in all respects to those seen prior to the 18th serial transfer of the virus in the first series of experiments and described in an earlier paper (2). At this writing the virus in the second series of experiments has reached its 12th passage in domestic rabbits and it still induces large fibromata when injected subcutaneously, while changed virus of the first series of experiments, carried in parallel, has continued to produce only a scant inflammatory reaction when similarly administered. It thus appears that the change in the type of lesion produced was due to an alteration in the fibroma virus occurring during its 17th or 18th serial passage and not to any variation in the host.

Attempts to Restore Fibroma-Producing Properties to the Changed Virus

Since the natural host of the fibroma virus is the cottontail rabbit (genus Sylvilagus) (2) it seemed possible that its prolonged passage through a foreign host, the domestic rabbit (genus Oryctolagus), might be responsible for its modification. Instances in which viruses have been modified by transfer through unnatural hosts are well known. The question arose as to whether the change, in the case of the fibroma virus, was an irreversible one. Would passage of the changed
virus through cottontail rabbits restore its ability to induce fibromata when administered subcutaneously? To answer this question, virus of the 21st, 25th, and 31st serial passages was transferred intratesticularly to cottontail rabbits. The 21st passage virus was transferred through only 1 cottontail rabbit while the 25th and 31st passage viruses were submitted to 2 serial cottontail rabbit transfers. They were then carried back to domestic rabbits. In the first experiment the virus, after a single cottontail rabbit passage, induced large fibromata at the subcutaneous sites of inoculation in domestic rabbits of the first 2 serial passages. The inoculated testicles were diffusely infiltrated with fibroblast-like cells and the histological picture was identical in all appearances with that observed in rabbits infected with the original fibroma virus (2). In the 3rd domestic rabbit passage, however, the lesions both in the testicles and subcutaneous tissue were again composed largely of lymphocytes and large mononuclear cells with scattered islands of fibroblasts, indicating a reversion to the changed virus type of reaction.

The other two experiments, in which the changed virus was submitted to two serial transfers through cottontail rabbits, yielded similar results. In one of these experiments the virus was transferred only once in domestic rabbits and yielded lesions at all sites of inoculation which histologically appeared to be purely fibromatous. In the remaining experiment the domestic rabbit infected with virus from the 2nd passage cottontail rabbit developed typical fibromatous lesions wherever inoculated, but in the 2nd domestic rabbit passage a reversion to the inflammatory type of reaction was observed. The cellular reaction in the testicles of all cottontail rabbits used in these experiments was composed predominantly of lymphocytes with scattered islands of fibroblast-like cells.

These experiments demonstrated that the change in the fibroma virus observed in this laboratory was at least partially reversible and that the mere passage of the virus back through cottontail rabbits was sufficient to restore temporarily its fibroma-producing properties.

Attempts to Restore Fibroma-Producing Properties to Andrewes' Inflammatory Virus

In June of 1934, Andrewes sent me his inflammatory strain of the fibroma virus (designated IA in the preceding paper (1)). It has been passed serially in this laboratory 11 times through domestic rabbits and has induced only inflammatory lesions when injected intracutaneously or intratesticularly. Unlike my own changed virus, Andrewes' virus gives rise to no fibroblast-like cells in the inoculated testicle; the cellular reaction is limited to lymphocytes, large mononuclear cells, and occasionally a few leucocytes. It is thus even more altered from its original character than is my changed virus.
Since its receipt, Andrewes' virus has been submitted to 5 transfers through each of 2 series of cottontail rabbits, the animals of each passage being inoculated both subcutaneously and intratesticularly with testicle suspension prepared from the preceding passage. Subcutaneous injections caused no reaction in most instances but occasionally a scant thickening was observed. Histologically, the reaction was composed entirely of lymphocytes and large mononuclear cells. The inoculated testicles showed evidence of infection at each passage. After an incubation period of from 5 to 8 days they felt abnormally firm, but did not increase in size as did testicles of cottontail rabbits inoculated with the original fibroma virus. On the contrary, where only one testicle was inoculated, it tended to be smaller than the uninoculated testicle, when the animal was autopsied on from the 12th to 15th day after infection. At autopsy the inoculated testicle appeared to be acutely inflamed. It was usually a mottled purplish red, and blood vessels in the tunica albuginea were tortuous and widely dilated. On cut section considerable serosanguineous fluid exuded, and the testicular tissue itself appeared soft and dark red in color. This picture was in marked contrast to that exhibited by testicles of cottontail rabbits infected with the original fibroma virus in which the cut surface was firm, white or pinkish white, and fibroma-like in appearance.

Histologically the testicles of cottontail rabbits infected with Andrewes' inflammatory virus resembled those of similarly infected domestic rabbits. The spaces about the seminiferous tubules were densely packed with lymphocytes and mononuclear cells, the tubule cells were in some areas necrotic, and occasional foci of leucocytic infiltration were encountered. No fibroblast-like cells, so common in testicles infected with the original fibroma virus, were to be seen.

No change in the type of lesion produced by the virus of either series of cottontail rabbit passages has been observed. Testicular lesions have been persistently inflammatory in character. 5th passage virus from both series, as well as that from earlier passages, has been transferred subcutaneously, intracutaneously, and intratesticularly in domestic rabbits, and in every instance the lesions have been inflammatory in character. The virus has failed to recover any of its ability to produce fibromata in either cottontail or domestic rabbits. The inflammatory virus has thus differed from my own changed virus in that cottontail rabbit passage has not re-endowed it with the property of inducing connective tissue hyperplasia at sites of inoculation in domestic rabbits. This difference in the two viruses will be discussed later (3).

**The Identity of Inflammatory Virus and Fibroma Virus**

Although the types of lesion produced in rabbits by the inflammatory and the original fibroma virus are distinctly different, cross-
immunity and cross-neutralization experiments described in the preceding paper (1) have indicated that the two viruses are immunologically identical. In this laboratory both cottontail and domestic rabbits have been tested for susceptibility to the original fibroma virus following recovery from infection with the inflammatory virus, and all were found to be completely resistant.

In earlier experiments (4) it was observed that domestic rabbits, which had recovered from infection with the fibroma virus, were usually resistant to the highly fatal Virus myxomatosis. An attempt was made to determine whether the inflammatory virus had the same effect. 26 days after infection with inflammatory virus, 6 domestic rabbits were inoculated subcutaneously and intratesticularly with Virus myxomatosis. 2 normal control animals infected in the same way died of typical infectious myxoma after 8 and 10 days. None of the 6 rabbits previously infected with inflammatory virus succumbed or showed any symptoms of generalized myxoma. 5 developed localized myxomatous lesions where inoculated, and 1 remained completely negative. This experiment indicated that the inflammatory virus bore a relationship to Virus myxomatosis similar to that shown by the original fibroma virus.

SUMMARY

A change in the type of lesion produced by the fibroma virus has been observed. At about its 18th serial transfer in domestic rabbits the virus lost its ability to induce fibromas when administered subcutaneously. Instead, lesions developing in either the subcutaneous tissue or in the testicle were predominantly inflammatory in character and partly composed of lymphocytes and large mononuclear cells in place of fibroblast-like cells. Andrewes, working with the same virus in England, has observed a somewhat similar change. Passage of my changed virus through cottontail rabbits results in a transient recovery of the capacity to produce fibromata, while similar passage of Andrewes' inflammatory virus is without effect.

BIBLIOGRAPHY