A Study of Three New Duck Variants of the Rous Chicken Sarcoma*

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(Received for publication October 22, 1946)

Past studies have disclosed the fact that the virus of the Rous sarcoma of chickens can be adapted to alien species of birds provided, first, that young or newborn individuals be injected with the tumor material (2, 3) and second, that this tumor had been grown in chickens of a certain age (4). By fulfilling these requirements, several other duck strains of chicken tumors have been obtained. Three of them, variants of the Rous sarcoma, will be here described because they show interesting features differing from those of the sarcoma strains previously obtained from the same Rous tumor.

The method followed consisted of injection into the breasts of newborn ducklings of 3 cc. of a cell suspension of the chicken tumor at 1:5 in saline, and of analogous passages into other ducks of the tumors induced in the foregoing passage, using in these passages the same or a much smaller inoculum. Filtrates were also used. The original chicken tumor, as well as duck tumors from different passages were also tested on chicks and chickens by injecting from 0.5 to 3 cc. of cell suspensions in the breast, or 1 cc. or more of filtrate in either the breast or the vein. Adaptation of the virus to ducks was first recognized by gross and microscopic changes in the primary tumors, by the appearance of general lesions, and by the ability to infect older ducks with tumor material.

The tumor grew in the first passage in 16 of the 18 ducklings injected and regressed only in one case. Signs of adaptation were manifest in 13 of the birds. The rather amorphous chicken tumor changed into a grape-like type of growth consisting of round, solid, well-encapsulated tumor nodules mingled with similar nodules but filled with blood. From these primary lesions generalization occurred, as shown by the presence of hemorrhagic lesions in liver, spleen, bone marrow, lungs, kidneys, and also by the presence of occasional tumors in lungs and spleen. The ducks died within 14 to 34 days after inoculation. Separate lines were started from 7 of the tumors and 3 of them were carried through 3, 4, and 8 passages in ducks, being then purposely discontinued. The other 4 lines were lost because of accidental death or because the ducks employed were too old. Growth was always successful whenever ducks aged 1 or 2 days were inoculated, while it was far more irregular in older ducks. In the course of the passages, the tumors and generalized lesions showed the same characteristics as in the first passage. Death occurred within 13 to 30 days. Microscopically, the tumors were very pleomorphic sarcomas, although a large, distorted type of cell was predominant. Collagen was scanty or absent. Periosteal and endosteal tumors were observed in one case. The hemorrhagic lesions were observed, in general, independent of neoplasia.

Starting from a duck tumor of the third passage, the strain was maintained through chicks and pullets in 3 successive passages. Twelve out of 16 birds injected with cell suspensions in the breast, and 5 out of 6 injected with filtrates in the same location developed tumors. However, generalization from these tumors was negligible since only 1 of the animals developed a metastasis in the liver. At the end of the passage through chickens the tumor grew successfully in 3 out of 4 ducklings inoculated.

Strain 14(d)11.—The age of the donor chicken was 20 months and it had been inoculated with the tumor.
In 5 different passages, cell suspensions were injected into 14 chickens from 2 weeks to 3 months of age, with growth ensuing in all but one case. Filtrates were injected into 10 chicks, with positive results in all. In 3 occasions these chick-grown tumors were maintained through these hosts for 4 and 5 passages by means of cell suspensions. Growth was always successful and was often followed by generalization of the same type as seen in ducks. Filtrates were also active. At the end of the chick passages the tumors grew in ducks just as well as before these passages.

**Strain 14(d)7.**—The age of the donor chicken was 14 months. The bird had been inoculated with the tumor 43 days before. Cell suspensions of this tumor were effective in all of 7 chicks injected, but filtrates proved to be inactive in 2 other chicks.

The tumor grew rapidly in 4 of the 7 ducks injected, adaptation being manifest in one of the birds by the same signs as seen in the 2 tumors previously described. From this tumor a line was obtained which was discontinued after 3 passages. From 2 other tumors, lines were also obtained; one lasted 4 passages, while the other is now in its 20th passage. The ducks of the first passage died within 14 to 35 days. Like strain 14(d)11 the tumor soon became amorphous and very invasive. Survival time shortened rapidly so that quite frequently the ducklings injected with suspensions of tumor cells died in 8 or 10 days with or without generalization and, in some cases, even within 5 days, sizeable tumors had already been present. Generalization in viscera was quite frequent, with a frank predominance of hemorrhagic lesions. In fact, this was the most active strain in this respect. Periosteal and endosteal growths were quite common after a few passages, and often the bones of all the extremities were affected. However, the most interesting feature shown by strain 14(d)7 was the induction of hemorrhagic lesions in the central nervous system of a rather large number of ducklings. These lesions appeared either as a generalization from a primary tumor or following intravenous injections of filtrates, and they were observed grossly unassociated with neoplasia. A detailed report of the phenomenon, together with other pertinent experiments, will be given in another publication. Microscopically, the tumors were pleomorphic, like those induced by strain 14(c). Collagen was also scant or absent, and mitoses were rare.

Despite its extreme malignancy, no growth or growth followed by regression was observed in 5 ducks of from 6 to 18 months of age inoculated with cell suspensions. In general, the tumor grew constantly and steadily in ducks of from 1 to 30 days of age, although periods of poor growth, even in these young hosts, were encountered.

In 7 different passages a total of 38 chicks and 6 adult chickens were injected with filtrates of neoplastic or hemorrhagic lesions. Positive results were obtained in 22 chicks and 4 chickens. Cell suspensions were constantly effective in both chicks and chickens. On two different occasions the duck-grown tumors were easily kept for 5 successive passages through chicks and chickens, provided the donor host was young. With tumors from old hosts, growth was achieved in only 7 of 29 chicks and chickens injected. Generalization in viscera occurred in chicks as in ducks the same as periosteal and endosteal tumors. It is of interest to point out that in the latter case new formation of

44 days before. This is the oldest chicken ever found furnishing a tumor very easily adaptable to ducks. Filtrates and cell suspensions of this tumor proved to be very active for chicks.

The tumor grew very rapidly in all of the 4 ducklings of the first passage, and signs of adaptation were clear in 3 of the birds. They died within 28 to 34 days with large primary tumors and a pronounced hemorrhagic disease of the liver, and one of these tumors was maintained in 4 passages through ducks, the line being then purposely discontinued. The fourth duckling of the first passage died 7 days after inoculation with a large, viscid growth very much like the original chicken tumor and it showed no generalization. In the second passage, this tumor grew rapidly in all the 6 ducklings injected, again without signs of adaptation. These signs, though, appeared at the third passage in the form of grape-like tumors, as in strain 14(e), and with the development of generalized hemorrhagic lesions and also tumors. Passages by means of cell suspensions or filtrates were continued and the line is now at its 20th passage. In the course of transplantation, the grape-like tumors soon disappeared to be replaced by rather amorphous, viscid growths of an extremely invasive type which rapidly infiltrated the skin and penetrated the abdominal cavity. Generalized hemorrhagic lesions with or without association with tumors were frequently observed in the viscera, and in one case there developed periosteal and endosteal tumors. Death occurred in from 10 to 20 days after inoculation with or without generalized lesions grossly visible.

Microscopically, the growths were rather uniform with long attenuated fibroblasts as the dominant cell type. Collagen was scarce or absent. Mitoses were rare. The hemorrhagic lesions in general were not associated with neoplasia.

The tumor always grew when ducks from 1 to 4 days of age were inoculated. It occasionally regressed in ducks 4 or 5 weeks old, and it grew but slowly and without inducing generalization in ducks 6 or 18 months old.
osteoid tissue was never observed in chicks or ducks as well.

Further study of the strain disclosed the following fact of interest: At the 19th passage, 2 chickens 8 months old were injected intradermally with filtrate of a duck tumor. One of the birds, killed 60 days later, showed a sarcoma in the injected site $4 \times 2 \times 2$ cm., conspicuous lung metastases, a large embryonal nephroma in the left kidney, and 2 smaller ones in the opposite kidney. The primary tumor showed histological signs of regression. Injection of cell suspensions of the primary tumor into 4 chicks and pullets resulted in tumors which later regressed, but injection of a similar material from the embryonal nephroma in another 4 chicks and pullets resulted in large sarcomas, one of which was maintained in chickens through 6 successive passages by cell suspensions and filtrates.

The other chicken injected with the filtrate of the duck tumor also showed a local sarcoma $4 \times 3$ cm. and lung metastases. The primary tumor was carried in chicks through 6 successive passages.

Leaving aside the point concerned with the significance of the kidney lesions, the point of interest is that the virus from the tumors of the 2 chickens had lost to a large extent its former capacity to infect ducks, for in each of the 2 serial passages through chicks, ducks were also injected with the following results: In the line from the embryonal nephroma, no growths were obtained in 20 ducks of from 1 to 30 days of age injected with cell suspensions or filtrates, and in the line from the local sarcoma, only 12 ducks a few days old out of 37 injected with cell suspensions developed tumors, but these growths were well localized and never induced generalized lesions.

**DISCUSSION**

In addition to individual characteristics, the 3 variants of the Rous sarcoma show some common traits which are different from those shown by all or some of the 6 variants of the same sarcoma obtained in 1941 and reported the following year (2).

The most important difference is that in the variants here described, despite the adaptation of the virus to ducks, their affinity for the original host, the adult chicken, was kept and it was possible to maintain the strains by passages through both chickens and ducks indifferently. 1 Other differences were the lack of

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1 This does not mean that the disease in chickens was the same as that induced by the original Rous sarcoma virus as attested, for instance, by the frequent development of periosteal and endosteal sarcomas in chickens injected with virus from strain 14(d)7 or by the absence of generalized lesions in those injected with virus from strain 14(e).

affinity of the virus for the skin and intestinal tract, the amorphous character and absence of collagen in the tumors, and the lack of production of osteoid tissue following the development in chicks of periosteal and endosteal tumors. Since both the strains of 1941 and those here described were obtained from the same strain of Rous sarcoma, these findings may still be another proof (4) of the variability of the tumor virus, a variability which is not manifested in routine passages through the homologous host but only when heterologous hosts are infected.

The lack of neoformation of osteoid tissue in the chicks that developed periosteal and endosteal tumors is of interest since in some of the 1941 strains it was observed that the bone condition *osteopetrosis gallinarum* (1-3, 7) arose as a late lesion in chickens infected with viruses inducing this neoformation of bone. Osteopetrosis was never observed in the chickens injected with viruses of the duck variants of the Rous sarcoma here studied, and in this respect these viruses behaved like those inducing some other spontaneous chicken tumors (5). These observations tend to support the idea that transmissible osteopetrosis is not a common response to many unrelated stimuli but rather a specific lesion associated with infection by leukosis and tumor viruses.

Strain 14(d)7 deserves additional comment. In the first place, it is the most malignant variant ever obtained, sometimes killing the host in as short a period as 5 days without visible generalization but with sizeable tumors. This property was shared to a certain extent by the other duck variants of both 1941 and 1945, and may be an indication of a toxic effect. Therefore, the process of variation has resulted in a pronounced increase in the virulence of the tumor virus. However, despite this virulence, strain 14(d)7 and the others as well are but little effective in older ducks, because of the development of an age resistance by these hosts. Strain 14(d)7 was also the most active in inducing hemorrhagic lesions, and stands as a unique example in the sense that it induced such lesions in the central nervous system of ducklings. Still another property of the strain is its pronounced activity for full-grown pigeons, in which hosts malignant tumors followed by generalization were produced by cell suspensions. These latter two properties will be dealt with in separate publications.

A phenomenon of interest, so far only observed with the same strain, 14(d)7, is that after tumors were produced in 2 full-grown chickens by means of filtrates, the virus evidently varied again in these hosts because it lost its power to infect ducks older than a few days, and at best produced in new-born ducklings only rare
and well-localized tumors, never followed by generalization. Indeed, events were as if the ducklings had been injected with the Rous tumor grown either in very old or in very young hosts (4). Therefore, it would seem that the virus reverted to a chicken type, but whether or not that virus was exactly the same as the original Rous virus cannot be said.

In both the tumors here studied and in those induced in 1941 by cell suspensions, the frequency of cases in which signs of adaptation were observed in the ducklings of the first passage can be taken as an indication of the number of virus units which have previously mutated or have been selected in the chicken tumor employed for inoculating the ducklings. In strains 14(e) and 14(d)11, practically all of the ducks of the first passage showed signs of adaptation of the tumor, whereas, in strain 14(d)7 as well as in some of the 1941 strains, signs of adaptation were not clear until the tumor had been carried in several passages through ducks. One can presume that during these passages the units of virus which had retained their original nature as inducers of the chicken tumor had been progressively eliminated.

Finally, what has been learned from all the duck variants of the Rous sarcoma so far studied emphasizes the fundamental instability of the viruses of avian cancer which, through a process of variation, can induce a practically limitless number of strains, each different from the others.

SUMMARY

Three new variants in ducks of the Rous sarcoma of chickens were obtained. Because they showed characteristics different from those of the variants previously studied, the strains are described in some detail. The most important difference was that the viruses of the strains here studied, despite becoming adapted to ducks, did not lose their affinity for their original host, the chicken. Also, the tissue affinities of the viruses, the amount of collagen in the tumors, and the reaction of bones of chicks to periosteal and endosteal tumors were different. The virus of one of the variants, strain 14(d)7, was of special interest for the following reasons: first, because after infecting adult chickens it lost its acquired affinity for ducks and reverted to a chicken tumor type; second, because it induced malignant tumors in pigeons of all ages; and third, because it showed an affinity for the central nervous system of young ducks.

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