Growth Curve and Distribution of Rous Sarcoma Virus (Bryan) in Japanese Quail¹

ROMAN J. PIENTA² AND VINCENT GROUPÉ

Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey, 08903

Received for publication 2 September 1967

On primary infection with the Bryan strain of Rous sarcoma virus (RSV), the growth curve of the virus in the brain of Japanese quail was similar to that observed in chicks and turkey poults. Infectious virus disappeared from the brain after inoculation. After an eclipse period during which no virus was detectable, infectious virus began to appear at 2 days and reached maximal titers in the brain samples at 7 days after inoculation. When Japanese quail were infected intracerebrally with RSV, relatively high titers of virus were recovered from brain tissue but not from liver, lung, kidney, or blood of moribund birds. Only tumors produced in the wing web of quail infected subcutaneously yielded high titers of virus. Other tissues yielded no virus, even though wing web tumors appeared as early as in chicks similarly infected. RSV could be propagated in the wing web of quail for at least 14 passages without any loss of infectivity. On the other hand, serial passage in quail brain resulted in a progressive loss of infectivity until virus was completely lost.

Reyniers and Sacksteder (10) were able to induce tumors in Japanese quail raised under germ-free or conventional conditions by infecting them with high doses of the Bryan strain of Rous sarcoma virus (RSV). Bryan RSV has also been shown to induce the formation of pocks on the chorioallantoic membrane of Japanese quail embryos (8) and foci in secondary cultures of quail embryo fibroblasts (2).

Later Rauscher, Reyniers, and Sacksteder (9) showed that the Bryan strain of Rous sarcoma virus could be serially propagated in the breast muscle or wing web of adult Japanese quail of the T-2 strain. The standard virus could not be serially passed in quail embryos unless it had first been passed 10 times in quail wing web. All attempts to isolate leukosis viruses from normal or experimentally infected adult quail or embryos were unsuccessful.

More recently, Shipman and Levine (12) observed that, although the several strains of Rous sarcoma virus they tested were able to induce tumors upon primary inoculation, only the Bryan and Harris strains could be serially passaged in the wing web. Their 10th passage of the virus was found to contain Rous-associated

helper virus, as detected by the interference test.

Previous studies with baby chicks and turkey poults showed that high titers of infectious virus could be recovered from a variety of tissues when the birds were infected with standard Bryan RSV (13).

Similar studies were undertaken with Japanese quail to determine the distribution of infectious virus after intracerebral or subcutaneous inoculation of standard Bryan virus.

MATERIALS AND METHODS

Virus. Stable, frozen, standard Rous sarcoma virus (RSV) of the Bryan strain prepared from chicken tumor tissue by differential centrifugation (6) was kindly supplied by Frank J. Rauscher of the National Cancer Institute and stored in a dry-ice chest at -70°C until needed. Dilutions of the virus were made in 0.85% sterile saline containing 2% inactivated normal horse serum and 100 units of penicillin G and 100 μg of streptomycin sulfate per ml. For subcutaneous inoculation, 0.1 ml of virus suspension was inoculated into the web of the left wing. To avoid leakage, the 0.75-inch (1.8-cm), 26-gauge needle fitted to a 1-ml tuberculin syringe was passed through the muscle and into the subcutaneous tissue of the wing web. Beginning with the 1st day after infection, each bird was examined daily for the appearance of tumors at the site of inoculation. For intracerebral inoculation, 0.02 ml of the virus inoculum was injected by means of a 0.25-inch (0.6-cm), 27-gauge needle. The birds were lightly anesthetized with ether before inoculation. Immediately prior to use, the tissue samples were thawed and homogenized in a mortar with

¹ A preliminary report of these data was presented at the 67th Annual Meeting of the American Society for Microbiology, New York, N.Y., 2 May 1967.

² Present address: Department of Pathology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Tex. 77025.

alundum and an amount of diluent sufficient to make a 10% suspension (weight/volume). The homogenates were clarified by centrifugation at 2,000 rev/min for 20 min in a refrigerated model PR-2 International centrifuge. The supernatant fluids were collected and used as the cell-free extracts.

Collection of sera and antibody titration. Blood was collected from the quail by cardiac puncture. The blood samples were allowed to clot at room temperature and stored overnight at 4 C. The serum which was then obtained by centrifuging each sample at 2,000 rev/min for 20 min was stored at -20 C until needed. All sera were inactivated by heating at 56 C for 30 min. To determine the antibody titer or virus-neutralizing capacity of the sera, expressed as the neutralization index (NI), equal volumes of serial 10-fold dilutions of standard Bryan RSV and serum diluted 1:10 were mixed and allowed to stand at room temperature for 45 min. Groups of seven embryonated eggs each were then injected with 0.1-ml amounts of each mixture. Control mixtures contained diluent or normal serum and appropriate dilutions of RSV. The neutralization index was calculated by subtracting the log number of pock-forming units (PFU) per milliliter of the virus-immune serum mixture from the log PFU per milliliter of the virus-control serum mixture.

Quail. Japanese quail of the T-2 strain were purchased from Shamrock Farms, North Brunswick, N.J. All quail were less than 3 weeks of age when inoculated with the virus. They were housed in standard brooder batteries previously fitted with 0.25-inch metal hardware cloth and were maintained on a diet of Turkey Startina (Ralston Purina Co., St. Louis, Mo.) and water *ad libitum*. To facilitate daily recording of tumor response and deaths, each bird was identified by a numbered metal band (National Band and Tag Co., Newport, Ky.), secured to the right wing web.

Assay for virus content. The virus content of tumors and other tissues collected for assay was done by using the modified pock count technique devised by Groupe et al. (3), which employed the chorioallantoic membrane of embryonated hen eggs. When necessary, tissue samples were stored at dry-ice temperature until assayed for virus content. The various tissue samples were homogenized as 10% suspensions in diluent supplemented with 1 mg of hyaluronidase (300 turbidity-reducing units/mg) per 100 ml. After centrifugation, the supernatant fluids were collected, and further 10-fold dilutions were made as needed. The diluted samples were then injected, in 0.1-ml amounts, onto the dropped chorioallantoic membranes of groups of 9-day-old embryonated eggs. Seven eggs were injected for each dilution. After sealing the holes in the eggs with collodion, the air sacs were returned to their normal position by placing the eggs in a vertical position with their blunt ends uppermost. The eggs were incubated at 38 C and candled for deaths 3 and 10 days after inoculation. Dead eggs were discarded, and all eggs alive at 10 days were chilled overnight at 4 C to kill the embryos. The chorioallantoic membranes were removed and rinsed in saline; the pocks on them were then counted. The virus titer of the inocula were

FIG. 1. Growth curve of Bryan strain of Rous sarcoma virus in Japanese quail brain and death rate of quail after intracerebral inoculation with 0.02 ml of standard virus.

calculated and expressed as the log number of PFU per gram of tissue. Standardized samples of Bryan RSV were included in each assay experiment as an internal control of the system.

RESULTS

Growth curve of RSV in the brain of Japanese quail. In the initial growth curve experiment, 75 nine-day-old quail of the T-2 strain were infected with a 10^{-1} dilution of standard Bryan RSV (CT-912) by inoculating 0.02 ml into the right cerebral hemisphere. The quail were then divided into two groups of 45 and 30 birds each, and beginning with the first day after infection two birds were sacrificed daily, at random, from one group. Their brains were collected, pooled, and subsequently assayed for virus content. The birds in the second group were not sacrificed, but were allowed to die from the disease. The growth curve of the virus in quail brain and the mortality rate are illustrated in Fig. 1. One can see that the growth curve obtained is similar to that obtained when chicks (4) or turkey poults (1) were infected intracerebrally with RSV. Infectious virus disappeared from the brain after inoculation. After an eclipse period during which no infectious virus was detectable, virus began to appear at 2 days and reached peak titers in the brain samples at 7 days, at which time quail in the second group began to become moribund. All quail in this second group were dead by 13 days.

Distribution of infectious virus. Previous studies with baby chicks and turkey poults (13) showed that high titers of virus could be recovered from a variety of tissues, namely, brain, kidney, liver, lung, and spleen, when RSV was injected intra-

cerebrally or subcutaneously into those avian hosts. For the sake of orientation, data excerpted from that report are summarized in Table 1. To determine the distribution of virus in Japanese quail after intracerebral injection, a group of 9-day-old quail were infected as previously described, but, in addition to brain, other tissues were collected and assayed for virus content. The results obtained are shown in Table 2. In this experiment, infectious virus was detectable in quail brain at 3 days and reached maximal

titers at 7 to 8 days. However, it is interesting to note that little or no virus was found in the other tissues examined, even in those quail dying from the disease. These results are quite different from those obtained with either baby chicks or turkey poults, where, as you may recall, most tissues contained substantially high titers of infectious virus.

The distribution of infectious viruses in quail after subcutaneous wing web inoculation of standard Bryan RSV was also studied. In this experiment, a group of quail were infected by injecting 0.1-ml amounts of a 10^{-2} dilution of Bryan RSV into the wing web of 9-day-old quail. It should be noted that tumors appeared in the wing web of all the quail by the 3rd day, but were not large enough to collect for assay until about 10 days after inoculation. Nevertheless, two birds were sacrificed daily for 15 days, and various tissues were collected and assayed for virus content. The results are summarized in Table 3. As when the intracerebral route of infection was used, it can be seen that infectious virus was recovered only from tissues at the site of inoculation. Tumor samples collected 10 to 15 days after inoculation contained appreciable amounts of virus, whereas little or none could be detected in most of the other tissues collected.

Serial passage of RSV in Japanese quail. During this study, attempts were made to passage serially the Bryan strain of RSV in the wing web or brain of Japanese quail. The wing web passage series was initiated by inoculating a 10^{-1} dilution of standard Bryan RSV (CT-912) into the wing web of recipient birds. Birds were examined

TABLE 1. *Distribution of virus in tissues of chicks and turkeys inoculated with Rous sarcoma virus (Bryan) (13)*

Route of inoculation ^a	Host tissue examined	Virus titer	
		In chicks	In turkeys
Intracerebral	Brain	7.2 ^b	5.3
	Kidney	2.4	5.0
	Liver	7.0	6.7
	Lung	7.8	5.0
	Spleen	6.9	7.0
Subcutaneous	Wing tumor	7.0	7.5
	Brain	3.2	2.1
	Kidney	5.3	6.6
	Liver	7.0	5.1
	Lung	6.9	4.9
	Spleen	7.5	7.4

^a Lot CT-798, diluted to 10^{-1} ; 0.01 ml intracerebrally; 0.2 ml subcutaneously.

^b Log pock-forming units/gram. Birds sacrificed when moribund.

TABLE 2. *Distribution of virus in tissues of Japanese quail inoculated intracerebrally with Rous sarcoma virus (Bryan)*

Days after inoculation ^a	Virus titer (log PFU) in				
	Brain	Lung	Liver	Kidney	Blood
1	ND, — ^b	ND, ^c —	1.6, —	ND, —	ND, —
2	—, —	—, —	—, —	—, —	—, —
3	2.0, 1.8	—, —	—, —	—, —	—, —
4	2.4, 1.6	—, —	—, —	—, —	—, —
5	2.8, 2.5	—, —	—, —	—, —	—, —
6	3.2, —	—, —	—, —	—, —	—, —
7	5.1, 4.9	2.3, —	—, —	—, —	—, —
8	5.1, 4.8	—, —	—, —	—, —	—, —
9	5.6, 3.4	1.3, —	—, —	—, —	—, —
10	4.9, 2.3	—, —	1.6, —	—, —	—, —
11	3.5, ND	1.9, ND	ND, —	1.6, ND	ND, —
13	3.6, 3.3	—, —	—, —	1.9, —	ND, —
15	3.7, 3.3	1.8, ND	ND, —	ND, —	ND, —

^a Lot CT-912 diluted to 10^{-2} ; 0.02 ml/bird.

^b Lower limit of assay = 1.0 log PFU.

^c Not done.

TABLE 3. Distribution of virus in tissues of Japanese quail inoculated subcutaneously with Rous sarcoma virus (Bryan)

Days after inoculation ^a	Virus titer (log PFU) in					
	Brain	Liver	Lung	Kidney	Blood	Tumor ^b
1	—, — ^c	—, —	—, —	—, —	—, —	
2	—, —	—, —	—, —	—, —	—, —	
3	1.6, —	—, —	—, —	—, —	—, —	
4	—, —	—, —	—, —	—, —	—, —	
5	—, —	—, —	—, —	—, —	—, —	
6	—, —	—, —	—, —	—, —	—, —	
7	—, —	—, —	—, —	—, —	—, —	
8	—, —	—, —	—, —	—, —	—, —	
9	—, —	—, —	—, —	—, —	—, —	
10	1.6, —	1.6, —	—, —	—, —	1.6, —	5.3, 5.5
11	—, —	—, —	—, —	—, —	—, —	
12	—, —	—, —	—, —	—, —	—, —	
13	—, —	1.9, —	—, —	—, —	—, —	4.9, 4.8
14	—, —	—, —	—, —	—, —	—, —	
15	—, —	—, —	—, —	—, —	—, —	3.3, ND ^d

^a Lot CT-912, diluted to 10⁻²; 0.1 ml/bird.
^b All quail positive by day 3. Tumors not large enough until day 10.
^c Lower limit of assay = 1.0 log PFU.
^d Not done.

FIG. 2. Serial passage of the Bryan strain of Rous sarcoma virus in the brain and wing web of Japanese quail.

daily and the appearance of tumors was recorded. Usually the first three tumors to appear were collected, and from these a 10% cell-free extract was prepared and used as the inoculum for the next passage. In this manner, the virus could be propagated easily for at least 14 passages without any apparent loss of potency. During the passage series, tumors began to appear 1 to 2 days after infection and generally all birds were positive by the 4th day (Fig. 2). Intracerebral passage of Bryan RSV in Japanese quail was less successful. As with the wing

web passage series, the brain passages were initiated with standard Bryan RSV (CT-912). Each of 30 quail was inoculated with 0.02 ml of a 10⁻¹ dilution of the virus. At this and each subsequent passage, three birds that were first to become moribund were sacrificed and their brains were collected and pooled. Cell-free 10% extracts were prepared and used for the next subsequent passage in 20 to 30 quail. Deaths were recorded, and the mean time to death was calculated for each passage. The results of the intracerebral passages are illustrated in Fig. 2. One can see that, when standard virus was used for the brain passage series, each passage resulted in a progressive loss of potency as evidenced by the increase in mean time to death. Cell-free extract prepared from the fourth brain passage failed to kill any quail. In an attempt to establish another brain passage series, a 10% cell-free extract of tumors obtained from the 9th quail wing web passage was used as the inoculum to initiate the series. However, similar results were obtained. After an apparent stabilization of potency in the first few passages, each subsequent passage resulted in a decrease in potency until, finally, no deaths occurred when a large number of quail were inoculated with cell-free brain extract from the seventh passage. Distribution of RSV after serial passage. Several samples collected during the serial passage of RSV in the wing web or brain of quail were col-

TABLE 4. *Distribution of virus in tissues of Japanese quail inoculated intracerebrally with second passage "quail-adapted" RSV*

Quail ^a no.	Virus titer (log PFU) in					
	Brain	Lung	Spleen	Kidney	Liver	Blood
4248	ND ^b	— ^c	—	—	—	—
4249	4.8	—	—	—	—	—
4250	5.0	—	—	—	—	—
4251	—	—	—	—	—	—
4252	3.0	—	—	—	—	—
4253	4.1	—	—	—	—	—

^a Three-week-old Japanese quail inoculated with 0.03 ml of a 10% brain extract from moribund birds.

^b Not done.

^c Lower limit of assay = 1.0 log PFU.

lected and assayed to determine whether the virus became more widespread in its distribution. Table 4 summarizes the results obtained when quail were inoculated intracerebrally with second-passage "quail-adapted" RSV. This virus was derived from standard Bryan RSV that had been passaged nine times in quail wing web and twice in quail brain, without any apparent loss in potency. The 10% cell-free extract killed 95% of the quail by 22 days when inoculated intracerebrally. However, Table 4 shows that, as with the original standard Bryan RSV, infectious virus could be recovered only from the brain of infected

TABLE 5. *Distribution of virus in tissues of Japanese quail inoculated intracerebrally with sixth passage quail brain RSV*

Days after inoculation ^a	Quail no.	Virus titer (log PFU) in			
		Brain	Lung	Spleen	Blood
7	4720	— ^b	—	—	—
	4721	—	—	—	—
	4722	—	—	—	—
	4723	—	—	—	—
	4724	—	—	2.8	1.6
	4725	—	—	—	—
10	4755	—	—	—	—
	4756	—	—	—	—
	4757	—	—	—	—
	4758	—	—	—	—
	4759	1.9	—	—	—
	4760	—	—	—	—
20	4789	—	—	—	—

^a Ten per cent extract of quail brain sample QB 4484-85; 0.02 ml/bird.

^b Lower limit of assay = 1.0 log PFU.

quail. None could be recovered from the lung, spleen, kidney, liver or blood samples collected from these moribund birds.

Similarly, cell-free extract from the sixth passage of RSV in quail brain was also tested. The data summarized in Table 5 show that, in this experiment, most tissue samples, including

FIG. 3. *Comparative capacity of standard Bryan Rous sarcoma virus, 13th quail wing web passage tumor extract, and 6th quail brain passage extract to induce wing web tumors or kill quail after subcutaneous (SC) or intracerebral (IC) inoculation, respectively.*

brain, collected at 7, 10, or 20 days after inoculation contained little or no infectious virus. Interestingly, however, as one can see from Fig. 3, although this sixth passage brain extract did not kill any quail after intracerebral inoculation,

TABLE 6. *Distribution of virus in tissues of Japanese quail inoculated subcutaneously with ninth passage quail tumor RSV*

Days after inoculation ^a	Quail no.	Virus titer (log PFU) in				
		Tumor	Kidney	Lung	Spleen	Blood
6	4225	ND ^b	— ^c	—	—	—
	4226	5.5	—	—	—	—
	4227	6.9	—	—	—	—
	4228	6.9	—	—	—	—
	4229	3.4	2.4	—	—	—
8	4238	4.6	—	—	—	—
	4239	6.8	—	—	2.8	—
	4240	4.6	—	—	—	—
	4241	2.9	—	1.6	—	—
	4242	5.0	1.8	2.0	—	—
10	4243	3.2	—	—	2.3	—
	4244	4.7	—	—	—	—
	4245	7.0	—	—	4.7	—
	4246	5.0	1.6	—	3.2	—

^a Ten per cent extract of ninth passage quail tumor sample QT-4024-25; 0.1 ml/bird.
^b Not done.
^c Lower limit of assay = 1.0 log PFU.

it still had the capacity to induce wing web tumors in 100% of a group of quail by 13 days. In contrast, cell-free extract obtained from the 13th wing web passage had the capacity both to kill birds after intracerebral inoculation and to induce tumors in 100% of quail inoculated subcutaneously.

When quail were inoculated subcutaneously with cell-free extract obtained from the 9th passage of RSV in quail wing web, high titers of infectious virus could be found only in tumors at the site of inoculation. Occasionally, low titers of virus could be found in other tissues, notably the spleen. These data are summarized in Table 6.

Production of antiviral antibodies. To determine whether antiviral antibody was produced, various dilutions (10^{-1} , 10^{-4} , 10^{-6}) of standard Bryan RSV (lot TV-14) were inoculated into the wing web of groups of 2- to 3-week-old quail. At various times after inoculation, tumors from individual birds were collected and assayed for virus content. Concomitantly, serum from the same bird in each case was tested for its capacity to neutralize standard Bryan RSV inoculated onto the chorioallantoic membrane. The results are illustrated in Fig. 4, where it can be seen that, at all levels of initiating dose of virus, individual tumors contained highest titers of virus when they were collected 1 to 2 weeks after infection. Antibody levels were low at this time. Later, as antibody levels increased, there was a corre-

FIG. 4. *Virus content of tumors and production of antibodies in Japanese quail after subcutaneous inoculation of standard Bryan Rous sarcoma virus.*

FIG. 5. Production of antibodies in Japanese quail after intracerebral inoculation with fourth passage brain extract.

sponding decrease in the virus content of individual tumors. This is best seen in the cases where the quail were inoculated with the 10^{-6} dilution of the standard virus and tumors were collected from 2 to 8 weeks later. It should be noted that, in several cases, both circulating antibody and high titers of virus were present in the same bird. During one of the experiments in which standard Bryan RSV was passaged in quail brain, samples of brain tissue were collected and tested for virus content and their sera were tested for antiviral antibody activity (Fig. 5). In this experiment, none of the corresponding brain extracts from birds that provided the sera contained any detectable virus. This may be due to the low potency of the extract employed. One will recall from Fig. 2 that samples from the fourth intracerebral passage killed no quail. Nevertheless, low levels of antiviral antibody could be detected in the sera of birds inoculated with this material and sacrificed from 23 to 51 days after inoculation.

DISCUSSION

Several reports in the literature have described the growth of Bryan RSV in the wing web and brain of baby chicks or turkey poult (1, 4, 5, 13, 14). Spencer and Groupé (14) observed that the injection of birds with large doses of Bryan RSV resulted in the induction of tumors at the primary site of inoculation, i.e., brain or wing web, followed later by the formation of metastatic tumors in various tissues of the infected host. The presence of a high-titered viremia and the sequence of *in situ* changes leading to malignancy, observed in various tissues studied, prompted the authors to conclude that direct viral action was at least one major mechanism responsible for the formation of metastatic

tumor foci. When they injected large doses of Bryan RSV intracerebrally or subcutaneously into baby chicks or turkey poult, high titers of virus could be recovered from tissues that showed gross or histological evidence of malignancy. In addition, however, in many cases, appreciable amounts of virus could be detected in tissues that were apparently free from tumors when examined grossly or histologically.

The data described in this report show that the Bryan strain of RSV can be serially propagated in the wing web but not in the brain of the T-2 strain of Japanese quail. Contrary to the results obtained with baby chicks and turkey poult (13), infectious virus can be found at the site of inoculation and tumor formation, but not in most of the other tissues examined. In this particular study, the tissues collected and assayed for virus content were apparently free from macroscopic tumor foci. However, no histological sections of these tissues were examined and, therefore, metastatic tumors too small to be seen with the naked eye may have been overlooked inadvertently. Serial passage of the virus in quail wing web did not substantially result in an increase in the titer of virus in various tissues of the infected quail, nor did it result in virus progeny that could be more readily passaged in quail brain. The data show that neutralizing antibody can be detected in the sera of quail infected subcutaneously or intracerebrally with the virus. Although the presence of antibody might account for the absence of virus in tissues collected several weeks after inoculation, it would appear more likely that other factors are responsible for the absence of detectable virus in various tissues collected before 2 weeks, and for the failure of Bryan RSV to propagate serially in quail brain. It has been shown that the production of mature infectious Bryan RSV particles is contingent upon the presence of a helper virus, Rous-associated virus (6, 11). It also has been reported that normal Japanese quail and quail embryos of the T-2 strain are free from or refractory to infection with some avian leukosis viruses (9), suggesting that the helper virus, which may be one of several leukosis viruses, may not replicate efficiently in this species. Hence, one might possibly explain the inability to propagate Bryan RSV in quail brain serially by the progressive loss of the helper virus, resulting from its failure to replicate optimally at this site. At each passage, the brain extracts used for initiating the infection should then contain less of the helper, thus resulting in less infectious Bryan RSV so that eventually no birds die when inoculated with the cell-free brain extract. Such a loss in potency of the serially passaged brain extracts is illustrated in Fig. 2.

The lack of detectable infectious virus in various tissues of moribund birds might be similarly explained. Perhaps the initial coinfection with Bryan RSV and its contaminant helper permits the initiation of tumor formation and virus replication. However, because of the subsequent loss of helper below a minimal threshold level, release of sufficient mature infectious virus needed for secondary infection and tumor formation at other distant sites does not occur. That Bryan RSV can easily be passaged in the wing web of Japanese quail may be due to the ability of the helper virus to replicate more efficiently at this particular site. In this regard, Shipman and Levine (12) have reported the presence of a Rous-associated helper virus in samples of Bryan RSV passaged 10 times in quail wing web. One will also recall from Fig. 3 that, although the sixth quail brain passage extract failed to kill any birds when injected intracerebrally, tumors were produced in 100% of the quail inoculated subcutaneously with this same extract.

In the studies presented here, we did not test for the presence of a helper virus in our samples, nor did we preinfect the quail with helper virus or add it to the brain passage samples in an effort to increase their oncogenic potency.

ACKNOWLEDGMENTS

We are indebted to Domenica Bucolo for her able assistance.

This investigation was supported by Public Health Service grants CA-04313 and CA-05175 from the National Cancer Institute.

LITERATURE CITED

1. DUNKEL, V. C., F. J. RAUSCHER, AND V. GROUPÉ. 1964. Further studies on Rous sarcoma virus. Experiences with virus-host-tumor interactions in turkeys. *Natl. Cancer Inst. Monogr.* 17:179-200.
2. FREEMAN, G. 1963. Focus formation by Japanese quail cells infected with Rous sarcoma virus. *J. Natl. Cancer Inst.* 31:761-767.
3. GROUPÉ, V., V. C. DUNKEL, AND R. A. MANAKER. 1957. Improved pock counting method for the titration of Rous sarcoma in embryonated eggs. *J. Bacteriol.* 74:409-410.
4. GROUPÉ, V., AND F. J. RAUSCHER. 1957. Growth curve of Rous sarcoma virus and relationship of infecting dose to yield of virus in chick brain. *J. Natl. Cancer Inst.* 18:507-514.
5. GROUPÉ, V., F. J. RAUSCHER, A. S. LEVINE, AND W. R. BRYAN. 1956. The brain of newly hatched chicks as a virus-host system for biological studies on the Rous sarcoma virus (RSV). *J. Natl. Cancer Inst.* 16:865-875.
6. HANAFUSA, H., T. HANAFUSA, AND H. RUBIN. 1963. The defectiveness of Rous sarcoma virus. *Proc. Natl. Acad. Sci. U.S.A.* 49:572-580.
7. MOLONEY, J. B. 1956. Biological studies on the Rous sarcoma virus. V. Preparation of improved standard lots of the virus for use in quantitative investigations. *J. Natl. Cancer Inst.* 16:877-888.
8. RAUSCHER, F. J., J. A. REYNERS, AND M. R. SACKSTEDER. 1962. Japanese quail egg embryo as a host for viruses. *J. Bacteriol.* 84:1134-1139.
9. RAUSCHER, F. J., J. A. REYNERS, AND M. R. SACKSTEDER. 1964. Response or lack of response of apparently leukosis-free Japanese quail to avian tumor viruses. *Natl. Cancer Inst. Monogr.* 17:211-229.
10. REYNERS, J. A., AND M. R. SACKSTEDER. 1960. Raising Japanese quail under germfree and conventional conditions and their use in cancer research. *J. Natl. Cancer Inst.* 24:1405-1421.
11. RUBIN, H., AND P. K. VOGT. 1962. An avian leukosis virus associated with stocks of Rous sarcoma virus. *Virology* 17:184-194.
12. SHIPMAN, C., JR., AND A. S. LEVINE. 1966. Selective response of the Japanese quail to various strains of Rous sarcoma virus. *J. Bacteriol.* 92:161-163.
13. SPENCER, H. J., AND V. GROUPÉ. 1962. Pathogenesis of virus-induced Rous sarcoma. I. Distribution of virus and tumor foci in chicks and turkeys. *J. Natl. Cancer Inst.* 29:397-419.
14. SPENCER, H. J., AND V. GROUPÉ. 1962. Pathogenesis of virus-induced Rous sarcoma. II. Dynamics of tumor development and viral growth patterns in chicks. *J. Natl. Cancer Inst.* 29:421-441.