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Distribution of Deoxyribonucleic Acid Complementary to the Ribonucleic Acid of Avian Myeloblastosis Virus in Tissues of Normal and Tumor-Bearing Chickens

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^{3}H-labeled 70*S* ribonucleic acid (RNA) from purified avian myeloblastosis virus (AMV) was used as a probe in deoxyribonucleic acid (DNA)-RNA hybridization experiments to detect the presence of DNA complementary to the AMV genome in various tissues from noninfected normal chickens and from chickens infected with AMV. There was a remarkable constancy in the average cellular concentration of virus-specific DNA found in every tissue from the same uninfected chicken, and even in different chickens from the same strain. In contrast, different tissues from chickens bearing AMV-induced kidney tumors (embryonal nephromas) revealed an unequal distribution in the average virus-specific DNA content per cell. The increase was limited to tumor cells and to tissues that contain target cells for AMV, i.e., red blood cells, kidney cells, and possibly leukocytes. The red blood cells from AMV-infected chickens suffering from acute myeloblastic leukemia, although producing no virus, contained as many viral genome equivalents per cell as did leukemic myeloblasts known to produce large quantities of AMV. An increased viral DNA content was observed in the target cells of chickens that did not show any sign of tumor formation 6 months after infection with AMV. This study demonstrates that vertically transmitted viral DNA is uniformly and stably distributed among all tissues of the offspring, but that horizontal infection after hatching results in an increase in viral DNA content only in some dividing, target tissues that may or may not give rise to neoplasias.

Even though avian myeloblastosis virus (AMV) can replicate in a wide variety of embryonic or adult chicken cells after infection in ovo or in vitro, the expression of its oncogenic potential is limited to specific target cells both in the chicken and in vitro (1, 8, 10, 20, 29). After infection, these target cells give rise to a definite class of highly differentiated neoplasias: myeloblasts and their precursors which give rise to acute myeloblastic leukemia; erythroblasts and their precursors which account for acute anemia and occasionally erythroid leukemia; embryonic kidney cells which give rise to embryonic nephromas; osteoblasts and osteoclasts which cause an abnormal bone formation after destruction of normal bone (osteopetrosis); and lymphoid cells which cause lymphomatosis (1, 2, 8, 10, 16). All these neoplasias are induced if chicken embryos are infected, but if newly hatched chicks are infected, osteopetrosis is not induced, whereas myeloblastosis, erythroid disorders, and nephromas are induced less efficiently. The embryonic kidney tissue that gives rise to nephromas after infection with AMV supposedly persists in the kidneys only for a few weeks after hatching (17), and within 4 to 5 weeks after hatching kidney tumors can no longer be induced even with large doses of AMV (3).

There is a decrease in susceptibility to all forms of neoplasias as well as a change in the type of tumors that develop as the age of the chick increases (3, 10, 14, 15, 23). Resistance to tumor induction follows closely the maturation of the immune mechanism of the chicken, specifically the maturation of the Bursa-dependent lymphoid

system (3). Embryos are 100% susceptible, 8-week-old chickens are 99% resistant, and intermediate ages show intermediate states of susceptibility (3). Therefore, at least two factors play a role in the induction of neoplasias by AMV: immunity of the chicken and availability of target cells.

AMV and the other avian ribonucleic acid (RNA) tumor viruses (oncornaviruses) replicate their RNA genome via a deoxyribonucleic acid (DNA) intermediate (see references in 6 and 22, 26, 27).

It appears also that the proviral DNA can become permanently established in a cell by integration into host cell DNA (7, 22). All the apparently normal chicken embryos that have been tested contain long deoxynucleotide sequences that are homologous to the RNA of avian oncornaviruses (4, 7, 11, 13). Some of these normal chickens must contain information for an entire viral genome since oncornavirus production can be induced by various physical and chemical agents in explanted normal cells (30). Consequently, there are at least two classes of viral DNA in infected chickens: (i) viral DNA of unknown origin (which may represent the entire copy of an oncornavirus genome) that is vertically transmitted from parents to progeny, and (ii) viral DNA that represents a replicative intermediate acquired after experimental infection with an oncornavirus. A knowledge of the role of these two classes of viral DNA and of their interaction, if any, is required for our understanding of viral oncogenesis. As an approach to this problem, the present study was undertaken (i) to investigate the distribution of vertically transmitted viral DNA in various tissues of normal chickens and (ii) to determine the increase in viral DNA and its tissue distribution in chickens infected with AMV. AMV was injected into 1-day-old chicks, and the cellular concentration of viral DNA was determined in different organs of both 3-month-old chickens that developed kidney tumors and 6-month-old chickens which were still apparently healthy. Our findings demonstrate the presence of the same average cellular concentration of vertically transmitted oncornavirus DNA in every tissue of uninjected normal chickens, and indicates that after injection of AMV into 1-day-old chicks there is an increase in the cellular concentration of DNA complementary to AMV RNA that is restricted to specific target and tumor cells.

MATERIALS AND METHODS

Virus. The AMV BAI strain A, subroup B, was used.

Chickens and chicken embryos. Six apparently healthy 3- to 4-month-old adult hens, five White Leghorn (WL) and one Black Minorca (BM), were purchased from Desert Poultry Co., Baldwin Park, Calif. All untreated hens were sacrificed on the day of arrival. Embryonated eggs of the WL strain, cross K-137, were obtained from Kimber Farms, Pomona, Calif. One-day-old chicks were innoculated intraperitoneally with plasma from acutely leukemic chicks infected with AMV (3). Blood smears were prepared twice a week, and chicks or chickens suffering from acute myeloblastosis or showing signs of kidney tumors or lymphomatosis by abdominal palpation were sacrificed, and a postmortem examination was performed. Three non-egg-laying hens (no. 16307, 16322, and 16343) with obviously enlarged abdomens were sacrificed 102, 104, and 128 days postinfection, respectively. Blood smears indicated that these chickens were suffering from anemia and all three had unilateral or bilateral kidney adenocarcinomas. Over a 6-month period, 40 of 49 infected chickens from the same group developed AMV induced neoplasias. The background incidence of "spontaneous" leukosis in this strain of chickens was determined to be 8.2% by observation of untreated chickens for 8 to 12 months after hatching (3).

Six months postinfection, three apparently normal chickens were sacrificed, no. 16301 and 16308 on day 179 and no. 16325 on day 183. These chickens were healthy and had a normal blood picture, and postmortem examination revealed no abnormalities. Two were egg-laying hens (no. 16301 and 16308), and one was a rooster (no. 16325).

Isolation of leukemic myeloblasts and RBC. Myeloblasts and red blood cells (RBC) from acutely leukemic chickens were separated by sedimentation of heparinized leukemic peripheral blood at 500 rev/min for 15 min. The myeloblasts that had sedimented on top of the RBC were carefully removed, and each fraction was suspended in phosphate-buffered saline (PBS) and slowly centrifuged three times. The essentially homogeneous cell populations obtained in this manner were frozen at −70 C until extraction of DNA.

Isolation of various tissues. The normal chickens and those with kidney tumors were exsanguinated by cardiac puncture. The RBC were again isolated from the heparinized plasma by slow centrifugation, and both were stored at −70 C. There was a very faint, buffy-coat layer on top of the RBC after centrifugation, but unless specified it was left with the RBC. Specific normal organs were removed, washed with cold PBS and cut into small pieces of about ⅓ cm^3. The tissue pieces were rinsed in PBS until the supernatant fluid was no longer discolored and stored in 20-g samples at −70 C. Tumor tissue was similarly treated.

Extraction of DNA and RNA. The frozen tissue was thawed and homogenized in a Waring Blendor for 15 to 20 sec at a concentration of 5 to 10 g of wet tissue per 100 ml of extraction buffer (0.1 M NaCl; 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride, *p*H 8.5, and 0.001 M ethylenediaminetetraacetic acid. DNA was extracted and purified as previously described (13). Erythrocytes were lysed in glass-distilled water, and the nuclei were pelleted. The

nuclei were resuspended in distilled water and pelleted again. This procedure was repeated until the supernatant fluid was clear. The pellet was suspended in extraction buffer, homogenized in the Waring Blendor, and handled exactly as the other tissues. (Hybridization results were essentially the same whether DNA was extracted from intact RBC or from purified nuclei.) Only DNA species that gave an absorbance ratio (optical density 260:280 nm) greater than 1.70 were used.

Mouse embryo RNA and whole chicken embryo DNA were prepared as previoulsy described (13).

AMV RNA. ^{3}H-labeled 70*S* AMV RNA was prepared as previously described (13) except that only ^{3}H-labeled uridine (uridine-*5,6-^{3}H*; specific activity, 48.2 Ci/mmole; New England Nuclear Corp., Boston, Mass.) and cytidine (cytidine-*5-^{3}H*; specific activity, 25.4 Ci/mmole; Schwarz/Mann, Orangeburg, N.Y.) were used. Both labeled ribonucleosides were used at 10^{-6} M.

Virus production assay. Normal kidney cells and kidney tumor cells were plated at 10^7 cells per plastic culture dishes (10 cm; Falcon) in 10 ml of nutrient medium. When the plates were three-fourths covered, the medium was replaced with medium containing ^{3}H-uridine (10 μCi/ml). The plates were incubated for 24 hr, and the supernatant fluid was harvested and analyzed for labeled AMV virions as previously described (12). RBC from leukemic chicks were also tested in the same manner but at a concentration of 5×10^6 cells per ml of nutrient medium.

Infection of buffy-coat cells. After centrifugation of heparinized blood from nonleukemic chickens, the white blood cells formed a thin layer on top of the RBC. The buffy coat was suspended in nutrient medium at a concentration of about 2×10^6 white blood cells/ml and placed into plastic culture dishes. The cells soon attached to the surface of the dish and spread out. Two cultures were kept uninfected as controls, and four cultures were infected with AMV by the addition of 2 ml of nutrient medium containing 1 ml of cell-free plasma obtained from acutely leukemic chicks. After 1 hr of adsorption, 10 ml of regular nutrient medium was added to each plate. The cultures were refed 24 hr later and every 3 days thereafter. The cultures were kept under observation for 6 weeks for the appearance of conversion (8).

DNA-RNA hybridization. The method of hybridizing cellular DNA to ^{3}H-labeled 70*S* AMV RNA in a two-phase (filter-liquid) system has been previously described (5, 13). Mouse embryonic DNA was used as control to determine the amount of AMV ^{3}H-RNA nonspecifically bound; the averages ranged from 36 to 80 counts per min per mouse DNA filter in separate experiments and were deducted as background from the counts/min hybridized by the experimental filters. Each filter was counted for 10 min three times. Each filter contained, on the average, 25 μg of DNA as determined after counting hybridized radioactivity (13). The number of viral genome equivalents per cell genome in a given DNA preparation was determined by comparing the ratio of ^{3}H-labeled AMV RNA hybridized by that DNA to the amount of ^{3}H-labeled AMV RNA hybridized, under identical conditions, by DNA with a known concentration of viral genome equivalents (4, 7). This ratio is equal to the ratio of the viral DNA concentrations since the amount of ^{3}H-RNA hybridized at any given ^{3}H-RNA concentration depends only upon the viral DNA concentration under the conditions of hybridization used. The number of viral equivalents per cell genome in the reference DNA was determined at saturation levels of ^{3}H-viral RNA (7). Despite the fact that DNA from avian oncornaviruses, other than AMV, is present in normal chicken cells, this method remains applicable because RNA species from different avian oncornaviruses have base sequences that are indistinguishable by DNA-RNA hybridization (4, 7, 24, 28).

RESULTS

Constancy of average cellular concentration of DNA homologous to AMV RNA in different organs of normal adult chickens. The distribution of the average amount of viral DNA per cell in different organs removed from six healthy, adult hens is shown in Table 1. The five WL hens purchased from the same flock contained the same number of viral DNA equivalents per cell in their liver, lung, RBC, spleen, and kidney. The average cellular concentration of viral DNA in the five tissues tested was 3.0 genome equivalents for chicken WL-4, 3.3 for chickens WL-2 and WL-3, and 3.6 for chickens WL-1 and WL-5. The 24 tissues studied had a mean cellular concentration of 3.3 viral genome equivalents and a standard deviation of 0.32. Statistical analyses of the mean (*t* test) and of the variance (*F* test) have demonstrated that the cellular concentration of virus-specific DNA was the same in all the tissues of the same chicken and in all five WL that were tested. These findings are in agreement with those obtained with a large number of normal WL chicken embryos of the same strain (strain K-137); these embryos were found to have a mean of 3.2 viral genome equivalents per cell (4, 7). Therefore, the viral DNA present in the embryo persists throughout adult life and is evenly distributed among all the tissues of the chicken.

The one BM hen (BM-1) that was tested had an abnormally high number of viral DNA equivalents per cell (average of 7.1) in all five tissues that were tested. The high viral DNA content probably resulted from congenital infection by a nontransforming leukosis virus, but, unfortunately, no test was performed to determine whether this chicken was viremic. At autopsy, the BM hen appeared normal as did its peripheral blood picture. However, when the buffy-coat cells from the peripheral blood were tested in vitro for conversion by AMV, they were resistant to infection, as evidenced by the fact that they did not show conversion even 44 days after two separate infections with AMV. By comparison, the buffy-coat cells from WL-5, that

TABLE 1. *Hybridization of 70S AMV ³H-RNA to DNA from various tissues of normal adult chickens*[a]

Expt	Chicken	Counts per min hybridized per 100 μg of DNA[b]					
		Liver	Lung	RBC	Spleen	Kidney	Reference DNA
1	WL-1	667 ± 24 (3.5)[c]	699 ± 18 (3.7)	639 ± 56 (3.4)	686 ± 35 (3.6)	648 ± 29 (3.7)	1,191 ± 37 (6.3)
2	WL-2	511 ± 24 (3.1)	501 ± 31 (3.0)	584 ± 38 (3.5)	599 ± 36 (3.6)	525 ± 24 (3.2)	1,040 ± 45 (6.3)
	WL-3	517 ± 26 (3.1)	530 ± 37 (3.2)	567 ± 28 (3.4)	529 ± 31 (3.2)	564 ± 36 (3.4)	
3	WL-4	469 ± 57 (2.8)	502 ± 14 (3.0)	553 ± 59 (3.4)	512 ± 18 (3.1)	468 ± 24 (2.8)	1,037 ± 39 (6.3)
	WL-5	581 ± 37 (3.5)	509 ± 34 (3.1)	572 ± 58 (3.5)	689 ± 18 (4.2)	Not done	
	BM-1	1,113 ± 40 (6.8)	1,198 ± 61 (7.3)	1,090 ± 66 (6.6)	1,212 ± 37 (7.4)	1,180 ± 59 (7.2)	

[a] DNA was isolated from organs of six apparently normal 3- to 4-month-old adult hens, trapped on nitrocellulose filters, and hybridized at 70 C for 10 hr with 70S AMV ³H-RNA. Each vial contained five experimental filters from one organ in 1 ml of 4 × saline citrate with 0.05% sodium dodecyl sulfate, 3.0 mg of mouse embryo RNA, and 1×10^6 counts per min of AMV ³H-RNA (specific activity of 8.1×10^5 counts per min per μg). The counts per minute shown represent ³H radioactivity hybridized per 100 μg of DNA. Under these conditions, 24 to 28% of the total viral DNA hybridizable at saturation was hybridized to ³H-viral RNA. In each experiment, mouse DNA served as background control, and leukemic DNA with 6.3 viral genome equivalents per cell genome was used as reference DNA. The AMV genome was assumed to have a molecular weight of 12×10^6.

[b] Counts per minute ± standard deviation. Each filter was counted three times for 10 min each time.

[c] Average number of viral genome equivalents per cell. Calculated from the ratio of AMV ³H-RNA hybridized by the DNA of a given organ to the amount of AMV ³H-RNA hybridized, under identical conditions, by the reference DNA (4).

contained an average of 3.6 viral genome equivalents per cell, underwent typical conversion on day 6 after a single infection with AMV in vitro.

Increased viral DNA content in erythrocytes from leukemic chicks. To determine whether tissues other than leukemic myeloblasts also showed an increased viral DNA content after infection with AMV, RBC from leukemic chicks were examined. RBC were chosen because they are target cells and because pure RBC preparations can be easily obtained (see Materials and Methods), whereas other tissues may be infiltrated to varying extents by leukemic cells. AMV can induce proliferation of cells of the erythroid series when yolk sac cultures are infected in vitro (9). Also, AMV affects erythropoiesis since it induces acute anemia with a maturation arrest in the erythroid series. RBC from leukemic chicks, however, did not produce AMV or any detectable oncornavirions, if tested as described in Materials and Methods.

The virus-specific DNA content of DNA extracted from the RBC of leukemic chickens was compared to the viral DNA content of DNA extracted from leukemic myeloblasts (Table 2). Two pools of RBC from eight leukemic chickens and the RBC from one of two individual leukemic chicks contained as much viral DNA per cell genome as did leukemic myeloblasts; the latter contained from 4 to 7 viral genome equivalents per cell, with a mean of 5 equivalents. Uninfected chickens from this strain contain from 2 to 4 viral genome equivalents per cell with a mean of 3.2 equivalents.

Increased viral DNA content in specific tissues of chickens with AMV-induced kidney tumors. Chickens bearing kidney tumors (embryonal nephromas) induced by AMV not only provide a convenient source of well-defined localized tumor tissue, but also supply normal tissues that are free from tumor cells since the tumor does not metastasize. Consequently, 1-day-old chicks were injected intraperitoneally with AMV, and 3 months later three female survivors that had enlarged abdomens with a palpable tumor mass were sacrificed. Examination of their peripheral blood revealed that all three chickens had slightly immature (blue-gray) erythrocytes. In addition to the RBC, six apparently normal tissues (breast muscle, heart, liver, lung, spleen, and kidney) were

removed, and their DNA was tested for viral DNA content. The average number of viral genome equivalents per cell of these tissues is shown in Table 3. Tissues known to contain target cells for tumor induction by AMV showed an increase in the average cellular concentration of viral DNA, whereas non-target tissues did not. The normal cells—breast muscle, heart, liver, and left kidney—of chicken 16307 (which had a nephroma confined to part of the right kidney) contained a mean of 3.0 (standard deviation of 0.06) viral genome equivalents. The lungs, which may be considered a partial target tissue because they contain a large number of leukocytes and erythrocytes, showed a small, but significant (at the 0.01 level using the t test) increase in viral DNA content (4.1 viral equivalents per cell). The RBC contained 5.5 viral equivalents per cell, and the nephroma contained 6.1 equivalents per cell. Chicken 16322 which had bilateral kidney tumors did not show an increased viral DNA content in its lungs, but its RBC contained 5.2 equivalents and the two normal lower lobes of its right kidney that had a tumor in the top lobe, 7.8 equivalents; its normal tissues contained only 3.7 ± 0.26 equivalents. Chicken 16343 also had a significantly increased number of viral genome copies in its RBC (5.1 equivalents per cell) when compared to the non-target organs (breast muscle, liver, and spleen) which contained an average of only 3.8 viral DNA equivalents per cell. Both kidneys were entirely involved by the neoplasias, and, consequently, no normal kidney tissue was available for testing.

TABLE 2. *Increase in number of viral genome equivalents in the erythrocytes of leukemic chicks*[a]

DNA source	Counts per min hybridized per 100 μg of DNA[b]	Viral DNA equivalents per cell
Chick 16153 MB	2,146 ± 93	6.3
Chick 16153 RBC	2,448 ± 72	7.2
Chick 16136 MB	1,975 ± 34	5.8
Chick 16136 RBC	1,486 ± 67	4.4
Pooled leukemic RBC-1[c]	2,126 ± 51	6.3
Pooled leukemic RBC-2[c]	2,188 ± 52	6.5
Normal chicken embryo[d]	1,181 ± 59	3.5
Reference DNA	1,760 ± 74	5.2

[a] One-day-old chicks were infected intraperitoneally with AMV as previously described (10). Chick 16136 and chick 16153 were sacrificed by cardiac puncture after developing acute myeloblastic leukemia 23 and 26 days postinfection, respectively. The leukemic myeloblasts (MB) and red blood cells (RBC) were separated by slow-speed centrifugation. Hybridization and determination of viral DNA equivalents were done as in Table 1.

[b] Counts per minute ± standard deviation.

[c] Homogeneous preparations of RBC obtained by cardiac puncture from four leukemic chicks were used for each pool. Leukemic myeloblasts from different leukemic K-137 chicks contained on the average five viral genome equivalents per cell with a range from 4 to 7 equivalents.

[d] DNA was isolated from a whole, uninfected normal 11-day-old chicken embryo. The uninfected embryos from strain cross K-137 contain an average of 3.2 viral genome equivalents per cell, with a range from 2.1 to 4.5 equivalents.

Cells from the normal kidney and from the tumor of chicken 16307 were placed in culture and tested for production of C-type virions; neither type of cells were virus producers. Cells from the two kidney tumors of chicken 16322 did not produce virus either. The normal-looking kidney tissue from chicken 16322 failed to grow in culture, and the two tumors of chicken 16343 were not tested.

Increased viral DNA content in target tissues from apparently normal chickens that survived AMV infection. Some tissues from adult chickens that were still alive and well 6 months after infection with AMV were examined for viral DNA content. Three apparently normal chickens with a normal peripheral blood picture were chosen at random from among nine survivors in the group of chickens used in the previous experiment. This group consisted originally of 49 chicks injected on day 1 with a high dose of AMV (see Materials and Methods)—40 of which died from AMV-induced neoplasias. Two of the three chickens contained more viral DNA in their target tissues than in the other tissues tested (Table 4). Hen 16308 contained 4.7 viral equivalents per cell in the lungs and the RBC, and 5.2 equivalents in the kidneys; whereas its heart, liver, and spleen contained a significantly lower amount (at the 0.01 level using the t test) which averaged only 3.7 equivalents per cell. The viral DNA content in the lungs, RBC, and kidneys of rooster 16325 had also significantly increased (4.4, 4.0, and 5.4 equivalents, respectively) over the amount present in non-target cells which averaged 2.9 equivalents. The third chicken (hen 16301) did not show a significant increase in viral DNA content in any of the tissues that were tested.

These findings demonstrate that the cellular concentration of viral DNA can be increased in apparently normal target tissues by horizontal infection with an oncornavirus.

TABLE 3. *Hybridization of 70S AMV ^{3}H-RNA to DNA from different tissues of chickens with kidney tumors*[a]

Chicken no.	Avg no. of viral genome equivalents per cell[b]								
	Breast muscle	Heart	Liver	Lung	RBC	Spleen	Normal kidney	Kidney tumor no. 1	Kidney tumor no. 2
16307	3.0 ± 0.2	3.0 ± 0.3	3.1 ± 0.3	4.0 ± 0.1 4.2 ± 0.2	5.6 ± 0.2 5.4 ± 0.1	Atrophied[c]	3.1 ± 0.4 3.6 ± 0.2	6.1 ± 0.2	
16322	4.0 ± 0.2	3.7 ± 0.2	3.5 ± 0.2	3.6 ± 0.2 3.9 ± 0.3	5.0 ± 0.2 5.4 ± 0.2	3.4 ± 0.2	7.7 ± 0.2 7.8 ± 0.1	7.8 ± 0.2	7.8 ± 0.2
16343	3.6 ± 0.4 4.0 ± 0.1	Not done	2.9 ± 0.3	Not done	5.2 ± 0.4 5.4 ± 0.2 4.8 ± 0.1	4.4 ± 0.3 4.0 ± 0.2		10.6 ± 0.5 9.8 ± 0.3	8.0 ± 0.4 6.9 ± 0.3

[a] One-day-old chicks were injected intraperitoneally with AMV, and three that developed kidney tumors (embryonal nephromas) detected by abdominal palpation were sacrificed on day 102. Chicken 16307 had a nephroma confined to the upper lobe of the right kidney (kidney tumor no. 1); the other lobes of the right kidney and the entire left kidney (normal kidney) were not affected. Chickens 16322 and 16343 had bilateral kidney tumors. Chicken 16322 had a large cystic tumor involving the entire left kidney (kidney tumor no. 1) and a smaller cystic tumor of the top lobe of the right kidney (kidney tumor no. 2); the other two lobes of the right kidney appeared normal (normal kidney). Chicken 16343 had a teratoma involving the entire left kidney (kidney tumor no. 1) and a cystic tumor involving the entire right kidney (kidney tumor no. 2); there was no detectable normal kidney tissue. Hybridization was carried out as in Table 1.

[b] Mean of five filters ± standard deviation. Replicate determinations were carried out in separate experiments with a different set of filters and a different 70*S* AMV ^{3}H-RNA preparation (specific activity: 8.7×10^{5} counts per min per μg). Under these conditions, 24 to 26% of the total viral DNA hybridizable at saturation was hybridized to ^{3}H-viral RNA. The same reference DNA that contained 6.3 viral DNA equivalents per cell genome was used in every experiment and hybridized from 1,085 ± 38 to 1,191 ± 37 counts per min (mean of 1,118 counts per min) per 100 μg of DNA.

[c] At postmortem it was noted that the spleen of chicken 16307 was devoid of pulp.

TABLE 4. *Hybridization of 70S AMV ^{3}H-RNA to DNA from various tissues of apparently normal chickens that survived infection with AMV*[a]

Chicken no.	Avg no. of viral genome equivalents per cell[b]					
	Heart	Liver	Lung	RBC	Spleen	Kidney
16301	2.5 ± 0.4	2.5 ± 0.4	3.1 ± 0.1 3.2 ± 0.4	2.4 ± 0.3 2.6 ± 0.1	2.8 ± 0.3 2.8 ± 0.1	2.3 ± 0.2 2.2 ± 0.1
16308	3.8 ± 0.1	4.0 ± 0.1	4.7 ± 0.1 4.8 ± 0.2	4.7 ± 0.1 4.6 ± 0.3	3.5 ± 0.2 3.5 ± 0.1	5.0 ± 0.2 5.3 ± 0.3
16325	3.2 ± 0.4	2.9 ± 0.1	4.4 ± 0.4 4.3 ± 0.4	4.1 ± 0.4 3.9 ± 0.1	2.7 ± 0.3	5.4 ± 0.3 5.4 ± 0.2

[a] One-day old chicks were injected intraperitoneally with AMV, and three of the survivors that did not show any detectable signs of disease were sacrificed at 6 months. Hybridization was carried out as in Table 3. Duplicate determinations were carried out on some tissues with a different set of filters.

[b] Mean and standard deviation from five filters per determination.

DISCUSSION

Apparently all the cells from a normal, adult chicken contain the same number of avian oncornavirus genome equivalents. A total of 24 tissues from five 3- to 4-month-old WL hens were found to have an average of 3.3 viral DNA equivalents per cell. This is the same average cellular concentration as that found in each of 40 normal WL embryos of the same strain (6, 7). Presumably, every cell of the chicken embryo must also have contained the same amount of viral DNA. This indicates that this vertically transmitted viral DNA persists throughout the lifetime of the chicken and may be considered as part of its stable genetic information. Its nature and origin are unknown.

AMV-induced kidney tumor cells acquired two to three times the amount of viral DNA that is found in cells from uninfected chickens. This increase in number of viral genome equivalents is

similar to that found in leukemic cells and in cells transformed in vitro by avian oncornaviruses (7, 13). A similar increase in viral DNA content was also found in the RBC of leukemic chicks. These RBC are present in greatly diminished concentration (down to 1/10 the normal concentration) in the leukemic peripheral blood and are in late stages of maturation but do not produce AMV. Their increased viral DNA content may represent AMV DNA intermediates that resulted from the infection of erythroblasts, or other very immature erythroid cells, and can persist in chicken cells in the absence of virus production.

The chickens that were injected on hatching day and that developed kidney tumors showed, in different organs, a distribution of viral DNA quite unlike that observed in uninfected chickens. In the latter, vertically transmitted viral DNA was present at the same concentration in every tissue, but in the chickens infected with AMV the newly made viral DNA was detected only in some target tissues.

Congenital infection at an early stage of embryogenesis may give rise to a high cellular concentration of viral DNA in all the tissues. In addition, all the tissues of congenitally infected chickens may be permanent virus producers, whereas virus production is transient and limited to a few tissues if infection takes place after hatching (18, 25). In vitro culture conditions may resemble the conditions in the early embryo since all chicken embryonic cells cultured and infected in vitro acquire more viral DNA (5-13 equivalents) regardless of whether or not they become transformed (4, 7).

Therefore, only certain immature target cells that are dividing at the time of infection may permanently acquire new oncornavirus DNA. After infection, these target cells may remain normal or become tumor cells; they may or may not become virus producers. The cells that did not acquire additional viral DNA after infection may have been resistant to infection in situ, e.g., because of a lack of surface receptors for AMV or adverse metabolic conditions, or if infection took place and viral DNA was synthesized the latter could have been lost because of its inability to integrate into host DNA.

There was also an increase in the viral DNA content of some target cells even in chickens that did not show any sign of disease 6 months after infection. If the period of observation had been longer than 6 months, it is possible that these chickens might have developed tumors. Nevertheless, this experiment demonstrates that some target cells may acquire a relatively high concentration of viral DNA by infection with an avian leukosis virus after hatching and that these target cells may not give rise to tumors. This could lead to the sporadic virus production observed in some tissues, e.g., liver and pancreas of apparently normal chickens (19, 21), and suggests a possible origin for the "normal" complement of viral DNA in normal chickens. Horizontal infection might lead to the vertical transmission of the viral DNA in apparently normal chickens if the germ cells become infected.

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