JOURNAL OF VIROLOGY, May 1984, p. 301–308
0022-538X/84/050301-08$02.00/0

Vol. 50, No. 2

Specificity of Avian Leukosis Virus-Induced Hyperlipidemia

JEANNE K. CARTER† AND R. E. SMITH‡*

Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710

Received 11 July 1983/Accepted 7 November 1983

Rous-associated virus 7 (RAV-7) is a subgroup C avian leukosis virus which does not transform cells in vitro or carry an oncogene. When injected into 1-day-old hatched chicks, RAV-7 causes a low incidence of lymphoid leukosis after a latent period of several months. In contrast, infection of 10-day-old chicken embryos with RAV-7 leads to a disease syndrome characterized by stunting, obesity, atrophy of the bursa and the thymus, high triglyceride and cholesterol levels, reduced thyroxine levels, and increased insulin levels (Carter et al., Infect. Immun. **39**:410–422, 1983; J. K. Carter and R. E. Smith, Infect. Immun. **40**:795–805, 1983). Histopathological examination of tissues from affected chicks revealed an accumulation of lipid in the liver and an extensive infiltration of the thyroid and pancreas by lymphoblastoid cells. In the present investigation, the subgroup specificity of this syndrome was investigated. Other subgroup C avian leukosis viruses (transformation-defective B77, transformation-defective Prague C strain of Rous sarcoma virus, and RAV-49) caused stunting, infiltration of the thyroid and pancreas, increased liver weights, decreased thyroxine levels, and increased insulin levels, but they did not cause a uniform, profound increase in triglyceride and cholesterol levels. Avian leukosis viruses of subgroup A {myeloblastosis-associated virus 1 causing osteopetrosis [MAV-1(O)] and RAV-1}, subgroup B {MAV-2(O), MAV-2 causing nephroblastoma [MAV-2(N)], and RAV-2}, subgroup D (RAV-50), and subgroup F (ring-necked pheasant virus and RAV-61) did not cause a syndrome identical to that induced by RAV-7. All of the viruses examined induced some stunting and a reduction in thyroxine levels which correlated with the stunting. The two subgroup F viruses caused an infiltration of the thyroid which may have been secondary to severe lung involvement. We conclude that the RAV-7 syndrome is unique, particularly in the induction of a hyperlipidemia.

Avian retroviruses are classified into five subgroups based on susceptibility of chicken cells, interference, and neutralization (35, 36). Subgroup classification is a function of the envelope glycoproteins encoded by the viral *env* gene. Nonpathogenic endogenous viruses have been compared with exogenous and recombinant avian leukosis viruses to examine the correlation of subgroup with a variety of viral characteristics, including host range (35), growth rate (34), and definition of the host target cell for transformation by avian leukosis viruses in vivo (26, 27, 29). The conclusions of these studies are that regions of the viral genome near the *env* gene are involved in determining host range and growth rate but that they do not appear to influence the selection of a particular target for transformation.

Avian leukosis viruses are capable of inducing nonneoplastic disease in chickens, including anemia (23, 31), immunosuppression (32), and secondary hyperlipidemia as a result of endocrine dysfunction (5, 7). These diseases appear after a short latent period. Although some association with a viral subgroup is made in the appearance of anemia (31), the further correlation of a specific disease and a specific subgroup of avian leukosis virus has not been made.

The present study was initiated to examine the subgroup specificity of a virus-induced stunting and obesity syndrome in chickens. The results presented here establish that subgroup C avian leukosis viruses induce a lymphoblastoid infiltration of the thyroid and pancreas. This condition was uniquely induced by subgroup C viruses and was not induced by subgroup A, B, D, or F. The results suggest an involvement of the virus envelope in a specific nonneoplastic disease induced by avian leukosis viruses.

* Corresponding author.
† Present address: Department of Microbiology, School of Medicine, Vanderbilt University, Nashville, TN 37232.
‡ Present address: Department of Microbiology and Environmental Health, Colorado State University, Fort Collins, CO 80523.

MATERIALS AND METHODS

Viruses. The subgroup C viruses used in this study were transformation-defective B77 (*td*B77), *td* Prague C strain of Rous sarcoma virus (*td*PrC), Rous-associated virus 49 (RAV-49), and RAV-7. RAV-7 was endpoint purified three times before use. All subgroup C viruses were grown in chicken embryo fibroblasts, and titers of the supernatant stocks were determined before storage at −70°C. Viruses from various subgroups included two from subgroup A {myeloblastosis-associated virus 1 causing osteopetrosis [MAV-1(O)] and RAV-1}, three from subgroup B {MAV-2(O), MAV-2 causing nephroblastoma [MAV-2(N)], and RAV-2}, one from subgroup D (RAV-50), and two from subgroup F (ring-necked pheasant virus [RPV] and RAV-61), as well as the subgroup C virus RAV-7. All virus stocks except MAV-1(O) were supernatant fluids from infected chicken embryo fibroblasts. MAV-1(O) was serum passage 3 of biologically purified stock. Titers of the stocks were determined in chicken embryo fibroblasts, and the titer was indicated by plaque formation or the use of antisera to viral p27 in a fluorescent-antibody technique. The origin of the viruses and their subgroup designations are shown in Table 1.

Chickens. Fertile eggs from the experimental inbred SC White Leghorn line were obtained from Hyline International, Dallas Center, Iowa. Ten-day-old chicken embryos were inoculated by intravenous injection of a chorioallantoic vein (1). Subgroup C viruses were used at 10^2 infectious units per embryo in 0.1 ml of fluid. This virus dose of RAV-7 reproducibly induced disease in chickens (7). The study with five subgroups used 10^4 infectious units per embryo in 0.1 ml of fluid. The use of two levels of viral inoculum did not affect the results. After infection as embryos, chickens hatched with an efficiency of 53 to 100%. Chickens infected with different viruses were hatched and housed by subgroup in isolation rooms of the Animal Laboratory Isolation Facility

TABLE 1. Virus strains used

Strain	Subgroup	Source	Reference
MAV-1(O)	A	R. E. Smith	30
RAV-1	A	R. E. Smith	22
MAV-2(O)	B	R. E. Smith	30
MAV-2(N)	B	R. E. Smith	37
RAV-2	B	R. E. Smith	22
RAV-7[a]	C	C. Moscovici	12
*td*B77	C	C. Moscovici	33
*td*PrC	C	R. E. Smith	33
RAV-49	C	P. K. Vogt	12
RAV-50[b]	D	P. K. Vogt	2
RPV	F	P. K. Vogt	16
RAV-61	F	H. H. Hanafusa	18

[a] Bryan standard strain-associated virus.
[b] Schmidt-Ruppin-associated virus.

of the Duke University Medical Center, Durham, N.C. Uninfected chickens were hatched and reared in a separate room of the same facility. There were 6 to 12 chickens per experimental group.

Fertile eggs from the obese chicken line (OS), type B^1B^1, were obtained from R. K. Cole, Cornell University, Ithaca, N.Y. OS chickens were hatched and housed separately from the infected SC chickens in the isolation facility.

Histological examination. At predetermined intervals, chickens were fasted for 12 h and sacrificed by exsanguination via cardiac puncture. Body weights were determined, and livers were removed and weighed. Sections from the liver, pancreas, and thyroid were fixed in 10% buffered Formalin, dehydrated, embedded, sectioned, and stained with hematoxylin and eosin or methyl green pyronin. All histological sections were prepared by Experimental Pathology Laboratories, Inc., Raleigh, N.C.

Clinical chemistry. Levels of triglyceride and cholesterol in serum were determined by procedures previously described (5).

Hormone assays. Competitive radioimmunoassay determination of thyroxine (T_4) and insulin in serum was performed as previously reported (7). Determinations for a single time point were run on one assay so that a direct comparison of control and experimental levels could be made.

RESULTS

Subgroup C virus-infected and OS chickens. To determine whether all subgroup C avian leukosis viruses induced a similar disease syndrome, chickens were infected with 10^2 infectious units of *td*B77, *td*PrC, RAV-49, or RAV-7 as 10-day embryos. Chickens were necropsied and examined at 10, 20, 30, and 45 days posthatch (dph).

Histology. At 10 dph, all of the thyroid tissues from subgroup C virus-infected chickens were infiltrated with mononuclear cells which had large nuclei and prominent nucleoli. The infiltrating cells appeared to be lymphoblastoid. The extent of infiltration varied from 25 to 75% of the tissue (Table 2). The intact follicular epithelial cells were squamous to cuboidal, and the colloid lacked a uniform appearance. Involvement of the pancreas varied from slight (an increased density of islet cell nuclei) in *td*B77- and *td*PrC-infected chickens to severe (degeneration and infiltration of islets) in RAV-49- and RAV-7-infected chickens. The livers from all virus-infected chickens exhibited a mild, focal lymphocytic infiltration. Uninfected hatchmates showed no changes, whereas OS chickens showed mild vacuolization of the thyroidal colloid.

By 20 dph, the thyroids from all subgroup C virus-infected chickens and OS chickens contained extensive lymphoid cell infiltration, and germinal center formation was prominent in all by 30 dph. The infiltrating cell at 30 dph appeared to be more mature, and an increase in staining response to methyl green pyronin was noted, which was an indication of the presence of plasma cells. The presence of plasma cells was more pronounced in the thyroids of OS chickens. Pancreata at 20 and 30 dph showed marked infiltration, with germinal center formation in all subgroup C virus-infected chickens. The OS chickens showed no histological alteration in the

TABLE 2. Histology summary for subgroup C avian leukosis viruses

Virus	Histology[a] of the organs at dph:								
	10			20			30		
	Thyroid	Pancreas	Liver	Thyroid	Pancreas	Liver	Thyroid	Pancreas	Liver
Control	NRL	NRL	NRL	NRL	NRL	NRL	NRL	NRL	NRL
*td*B77	1–3+; colloid not uniform	1+; pycnotic nuclei	Focal hepatitis	2+	4+; germinal centers; degeneration	Focal infiltration	4+; germinal centers	Pancreatitis	Fatty
*td*PrC	3+	Pycnotic nuclei	Focal hepatitis	1–3+	2+; germinal centers	Slight fat	3–4+; germinal centers	2–4+; germinal centers	Fatty
RAV-49	2+	Pycnotic nuclei	Focal hepatitis	2–3+; germinal centers	2+	Slight fat; ductile proliferation	2–4+; germinal centers	3–4+; germinal centers	Fatty
RAV-7	1–2+	2+	Focal hepatitis	2–4+	2+	Slight fat; germinal centers	4+; germinal centers	3–4+; germinal centers	Fatty
OS	NRL colloid pale	NRL	NRL	1–3+	NRL	NRL	2+; germinal centers	NRL	Fatty

[a] NRL, No remarkable lesions; 1+, less than 25% infiltration; 2+, 25 to 50% infiltration; 3+, 50 to 75% infiltration; 4+, over 75% infiltration.

pancreas. The hepatocytes of all subgroup C virus-infected chickens and OS chickens showed decreased cytoplasmic staining and slight enlargement, probably due to increased fat accumulation at 30 dph, and the hepatocytes of RAV-7-infected chickens showed the greatest change. All uninfected chickens presented a normal histological picture (Table 2).

Hormone assays. T_4 and insulin levels in serum were examined to indicate an alteration of thyroid and pancreatic functions, respectively. The level of T_4 in the serum of uninfected chickens ranged from 1.97 to 1.1 μg/dl, with levels decreasing with age. At 10 dph, RAV-7- and *td*B77-infected chickens had significantly decreased T_4 levels (1.33 and 1.51 μg/dl, respectively), and the levels continued to drop in both groups to <0.5 μg/dl at 45 dph. Chickens infected with *td*PrC also exhibited a steady decline in T_4 levels from 1.59 μg/dl at 10 dph to 0.83 μg/dl at 45 dph, a level significantly below that in the uninfected chickens. RAV-49-infected chickens showed little change in T_4 levels at most times and showed a significantly lower level than that in the controls only at 20 dph. The OS chickens had levels of T_4 above those in the controls at 10 dph (2.17 versus 1.97 μg/dl), but levels of T_4 in OS chickens dropped to 0.73 μg/dl by 30 dph, a level significantly lower than that in control chickens (Fig. 1).

Insulin levels in *td*PrC-infected chickens were significantly higher than those in controls (10.7 versus 7.9 μU/ml) at 10 dph. Insulin levels in *td*PrC-infected chickens continued to increase to 15.9 μU/ml by 45 dph. The RAV-7-infected chickens showed increased levels of insulin, ranging from 10.0 to 17.4 μU/ml, whereas control chickens had insulin levels of 5.2 to 10.6 μU/ml. The insulin levels in RAV-7-infected chickens were significantly increased at 20, 30, and 45 dph (Fig. 1). By 45 dph, *td*B77- and RAV-49-infected chickens had significantly increased levels of insulin (9.21 and 10.47 μU/ml, respectively). The OS chickens did not show an increase in insulin levels at any time (Fig. 1).

Body weight. The weight of uninfected SC chickens was 48 g at 10 dph and increased to 441 g at 45 dph (Fig. 1). RAV-7-infected chickens were significantly stunted by 20 dph and weighed only 147 g at 45 dph. Chickens infected with *td*B77 were significantly stunted by 30 dph and weighed 200 g at 45 dph. Both *td*PrC- and RAV-49-infected chickens were significantly stunted at 45 dph, with weights of 249 and 272 g, respectively. The OS chickens were a larger strain of White Leghorn chicken, and a valid comparison of body weight with that of SC chickens was not possible (Fig. 1). However, the growth rate of the OS chickens slowed after 30 dph, and their physical appearance was similar to that seen in chickens with RAV-7 infection.

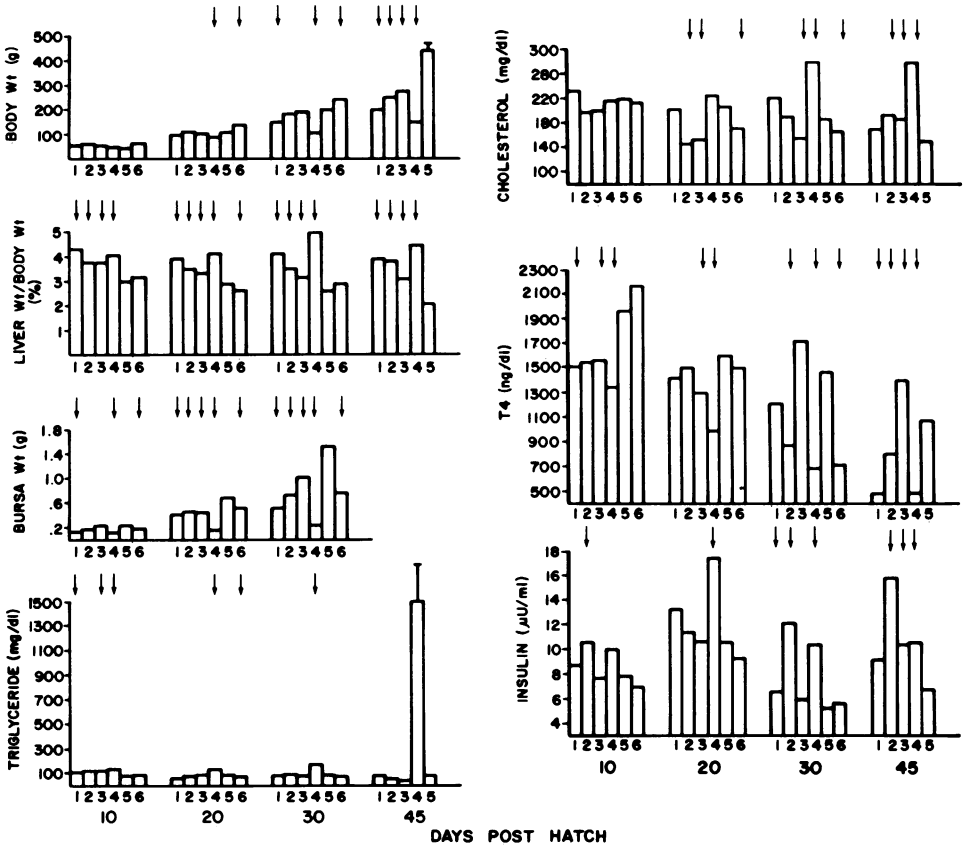

FIG. 1. Physical parameters of chickens infected with subgroup C avian leukosis viruses. Ten-day-old chicken embryos were infected with four subgroup C avian leukosis viruses. At 10, 20, 30, and 45 dph, chickens were sacrificed and examined. OS chickens which exhibited spontaneous autoimmune thyroiditis were examined at 10, 20, and 30 dph. Parameters measured were total body weight, relative liver weight expressed as a percentage of body weight, triglyceride in serum, cholesterol in serum, circulating T_4, and circulating insulin. (1) *td*B77; (2) *td*PrC; (3) RAV-49; (4) RAV-7; (5) uninfected controls; and (6) OS. The number of chickens examined for each group is as follows: *td*B77, 7; *td*PrC, 8; RAV-49, 8; RAV-7, 7; uninfected control, 9; OS, 9. ↓ denotes a significant difference from values obtained from uninfected SC control chickens at $P < 0.05$. The error bar expresses mean ± standard error of the mean.

Liver weight. Relative liver weights were expressed as liver weight/body weight × 100(%), which expressed both the decrease in body weight and the increase in actual liver weight. Uninfected control chickens had a relative liver weight of 2 to 3% of the body weight. All virus-infected chickens had relative liver weights of greater than 3.1% of the body weight at all times, and the relative liver weights were significantly increased at all times ($P \leq 0.05$). The largest relative liver weight was for RAV-7-infected chickens at 30 dph when the livers were 5.0% of the total body weight (Fig. 1).

Clinical chemistry. Triglyceride and cholesterol levels in serum were determined on fasting serum samples. The uninfected chickens had triglyceride levels of 68.2 to 90.5 mg/dl and cholesterol levels of 155 to 219 mg/dl. All test groups showed an increase in triglyceride levels at 10 dph, with a significant increase in *td*B77- and RAV-7-infected chickens. The increase was significant for RAV-7-infected chickens at 20 dph ($P \leq 0.01$), with a level of 124 mg/dl. By 45 dph, the level of triglyceride in the RAV-7-infected chickens had reached a mean of 1,500 mg/dl (Fig. 1).

Cholesterol levels in serum were normal in all groups until 20 dph, at which time chickens infected with *td*PrC and RAV-49 and OS chickens were significantly hypocholesterolemic (145.5, 156, and 174 mg/dl, respectively, compared with 208 mg/dl for the uninfected chickens; Fig. 1). At 30 dph, the level of cholesterol in RAV-7-infected chickens was 281 mg/dl, a level which was significantly hypercholesterolemic ($P \leq 0.005$). Chickens infected with *td*B77 showed an increase in cholesterol at 30 dph. RAV-49-infected chickens remained significantly hypocholesterolemic at 30 dph, with a level of 159.1 mg/dl ($P \leq 0.005$). At 45 dph, all virus-infected chickens had increased cholesterol levels in serum. At no point did OS chickens show elevated cholesterol levels (Fig. 1).

Subgroups A, B, C, D, and F. A comparison of diseases induced by four subgroups of avian leukosis viruses and one subgroup of pheasant viruses was made. Chicken embryos were infected with 10^4 infectious units of each virus, and chickens were necropsied at 35 dph, a time when the RAV-7-induced syndrome is advanced (7). Various disease states were found at necropsy and included osteopetrosis, liver lesions, blood cysts (hemangiomas), nephroblastoma, fibrosarcoma, angiosarcoma, and fatty liver (Table 3).

Histology. Sections of the thyroid, pancreas, and liver were Formalin fixed, processed, and stained with hematoxylin and eosin. The results are summarized in Table 4. At 35 dph, the thyroids from RAV-7-infected chickens were infiltrated by lymphoblastoid cells and had prominent germinal center formation. Few follicles remained intact. Subgroup A viruses, MAV-1(O) and RAV-1, showed slight (1+) thyroid infiltration which was focal. The infiltrating cell appeared more mature than that seen in RAV-7-infected tissue, and no germinal center formation was seen. Subgroup B viruses, MAV-2(O), MAV-2(N), and RAV-2, showed little or no infiltration of the thyroid. Thyroids from RAV-2-infected chickens had very slight (±) focal infiltration. RAV-50, a subgroup D virus, had a single focus of lymphoid infiltration which included a prominent germinal center. The subgroup F viruses, RPV and RAV-61, induced a more extensive focal infiltration of the thyroids. Both RPV and RAV-61 infection induced infiltration at 50 to 75% (2 to 3+) with multiple, prominent germinal centers (Table 4).

The pancreata from all virus-infected chickens except those infected with RAV-2 had various degrees of interstitial lymphocytic infiltration. This infiltration was slight for RAV-1 and extensive for MAV-1(O) and RAV-50. Germinal center formation was prominent in all affected pancreata. The infiltration in pancreata from RAV-7-infected chickens was in areas not defined as interstitial, and multiple perivascular and periductile germinal centers were present. An acute, focal pancreatitis was also evident in pancreata from RAV-7-infected chickens.

The livers of RAV-7-infected chickens had a diffuse fatty infiltration. Acute and subacute lymphocytic pericholangitis with numerous heterophils was apparent. None of the livers from any other subgroup showed diffuse fatty infiltration. Lymphocytic pericholangitis was apparent in chickens infected with MAV-1(O), MAV-2(N), and RAV-50. Germinal center formation was apparent with both RPV and RAV-61 infection. The livers from RAV-2-infected chickens showed little alteration. The most striking occurrence was the presence of numerous, discrete, sarcomatous areas in livers from MAV-1(O)-, RAV-1-, and RAV-50-infected chickens. In RAV-1-infected chickens, the sarcomatous mass became multicentric and replaced normal liver. A focal, acute necrotizing hepatitis was seen in MAV-2(N)- and RAV-50-infected chickens. No liver sections were available from MAV-2(O)-infected chickens, but previous investigations found no alteration in livers from MAV-2(O)-infected chickens (R. E. Smith, unpublished data) (Table 4).

Hormone assays. T_4 and insulin assays were performed by competitive radioimmunoassay. Uninfected chickens had T_4 levels of 1.89 μg/dl and insulin levels of 4.92 μU/ml. All virus-infected chickens showed a decrease in T_4 levels, with significant ($P < 0.05$) decreases (in micrograms per deciliter) as follows: RAV-7, 0.80; RAV-61, 0.90; RPV, 1.1; MAV-1(O), 1.27; MAV-2(O), 1.18; and MAV-2(N), 1.14. The least affected were RAV-50-infected chickens, with a level of 1.63 μg/dl. All T_4 levels except those in RAV-61-infected chick-

TABLE 3. Necropsy summary for various avian leukosis viruses[a]

Virus	Subgroup	No. infected	Disease (no. affected)	
MAV-1(O)	A	6	Osteopetrosis	(4)
			Anemia	(6)
RAV-1	A	12	Liver lesion	(2)
			Blood cysts	(1)
			Fibrosarcoma	(1)
MAV-2(O)	B	8	Osteopetrosis	(7)
			Blood cysts	(1)
MAV-2(N)	B	9	Nephroblastoma	(2)
			Blood cysts	(1)
RAV-2	B	10	Osteopetrosis	(1)
RAV-7	C	12	Fat accumulation	(10)
			Fatty liver	(8)
RAV-50	D	10	Anemia	(4)
			Osteopetrosis	(1)
RPV	F	10	Angiosarcoma	(1)
			Fibrosarcoma	(1)
			Nephroblastoma	(1)
			Blood cysts	(1)
RAV-61	F	9	Angiosarcoma	(4)
			Fibrosarcoma	(3)

[a] Findings at necropsy which was performed at 35 dph.

TABLE 4. Histology summary of various leukosis viruses

Virus	Subgroup	Histology[a]		
		Thyroid	Pancreas	Liver
Control		NRL	NRL	NRL
MAV-1(O)	A	1+; focal	3+; interstitial infiltration; germinal centers	Lymphocytic pericholangitis; discrete sarcomas
RAV-1	A	1+; mature lymphocytic, interstitial infiltration	1+; interstitial infiltration; germinal centers	Discrete to multicentric sarcomatous masses replacing normal liver
MAV-2(O)	B	NRL	2+; interstitial infiltration; germinal centers	Not available
MAV-2(N)	B	NRL	1+; focal interstitial infiltration; germinal centers	Bile retention; focal acute necrotizing hepatitis; lymphocytic pericholangitis
RAV-2	B	±	NRL	Foci basophilic cells
RAV-7	C	4+; germinal centers	3+; acute focal pancreatitis; multiple perivascular and periductile germinal centers	Diffuse fatty infiltration; acute and subacute lymphocytic pericholangitis
RAV-50	D	1+; single focus with germinal center	3+; interstitial infiltration; germinal centers	Acute necrotizing hepatitis; sarcomas; lymphocytic pericholangitis
RPV	F	2–3+; focal; prominent germinal centers	2+; interstitial infiltration; germinal centers	Germinal centers
RAV-61	F	1–3+; focal; prominent germinal centers	3+; interstitial infiltration; germinal centers	Germinal centers

[a] NRL, No remarkable lesions; ±, very slight infiltration; 1+, less than 25% infiltration; 2+, 25 to 50% infiltration; 3+, 50 to 75% infiltration; 4+, over 75% infiltration.

ens were significantly increased from the levels in RAV-7-infected chickens (Fig. 2). Insulin levels in RAV-7-infected chickens were significantly increased at 13.68 μU/ml. MAV-1(O)- and RAV-1-infected chickens showed significant increases in insulin levels, with 7.15 and 6.40 μU/ml, respectively. The remaining groups showed little alteration from control levels of insulin, and the levels in all groups were significantly decreased from that in chickens infected with RAV-7 (Fig. 2).

Body weight. Uninfected chickens in this study weighed an average of 296 g at 35 dph. All virus-infected chickens were significantly stunted ($P < 0.05$); RAV-7-infected chickens were the most stunted, with an average weight of 147 g. RAV-61-, RPV-, and MAV-1(O)-infected chickens showed a pronounced reduction in body weight, with average weights of 171, 171, and 173 g, respectively (Fig. 2).

Liver weight. Relative liver weight expressed as a percentage of body weight was 2.2% for uninfected chickens and ranged from 2.4 to 6.8% for the virus-infected chickens. RAV-7-infected chickens showed the largest relative (6.8%) and real liver weights for all groups (Fig. 2).

Clinical chemistry. Triglyceride and cholesterol levels in serum for uninfected chickens were 43.2 mg of triglyceride per dl and 168.8 mg of cholesterol per dl. MAV-1(O)-, RAV-1-, MAV-2(O)-, RPV-, RAV-61-, and RAV-7-infected chickens all had triglyceride levels significantly increased over levels in the uninfected controls. All virus-infected chickens, with the exception of RAV-7-infected chickens, had an average triglyceride level of below 90.0 mg/dl, significantly lower than the triglyceride level of 1,560 mg/dl in RAV-7-infected chickens. Cholesterol levels followed a pattern similar to the triglyceride levels, with cholesterol levels in all virus-infected chickens below 220 mg/dl and significantly lower than the levels of 396.4 mg/dl in RAV-7-infected chickens (Fig. 2).

DISCUSSION

When RAV-7 is injected into 10-day-old chicken embryos, the virus induces a rapid hypothyroidism and hyperinsulinemia which results in a secondary hyperlipidemia (7). Because this response is rapid (within 3 to 4 weeks posthatch) and appears at a high efficiency, the response of chickens to other subgroup C avian leukosis viruses under identical conditions was studied. The histological appearance of the thyroid, pancreas, and liver from chickens infected with *td*B77, *td*PrC, RAV-49, and RAV-7 could not be distinguished. The thyroids showed a lymphoblastoid infiltration at 10 dph, and the infiltration progressed to involve most of the thyroid, with apparent germinal center formation at 30 dph (Table 2). The pancreata from infected chickens showed a similar infiltration, although some variation in response was seen early in the course of the disease induced by the various subgroup C viruses.

The clinical parameters which were examined presented a more variable picture within the subgroup C avian leukosis viruses. Infection with three of the four subgroup C viruses examined eventually resulted in hypothyroidism (T_4 levels of below 0.9 μg/dl by 45 dph). The T_4 levels of RAV-49-infected chickens remained slightly elevated at 45 dph (Fig. 1). This result was surprising when the histological appearance of the

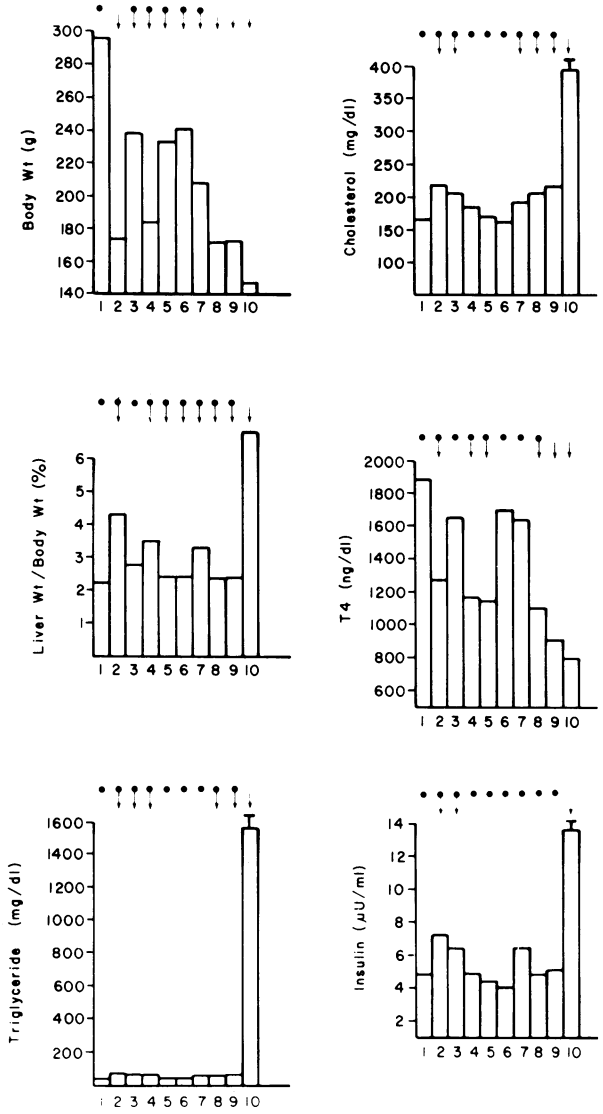

FIG. 2. Examination of five avian leukosis virus subgroups for RAV-7-induced changes. Ten-day-old chicken embryos were infected with nine avian leukosis viruses from subgroups A, B, C, D, and F. At 35 dph, chickens were sacrificed, and various parameters were measured. Parameters measured were total body weight, relative liver weight expressed as a percentage of body weight, triglyceride in serum, cholesterol in serum, circulating T_4, and circulating insulin. (1) Uninfected control; (2) MAV-1(O); (3) RAV-1; (4) MAV-2(O); (5) MAV-2(N); (6) RAV-2; (7) RAV-50; (8) RPV; (9) RAV-61; (10) RAV-7. The number of chickens examined and the necropsy summary for examination at 35 dph are shown in Table 3. ↓ = $P < 0.05$ compared with uninfected control; ● = $P < 0.05$ compared with RAV-7. All bars express means ± standard errors of the means.

thyroid was considered. Hashimoto's thyroiditis is marked by a diffuse infiltration of the thyroid by lymphocytic and plasma cells, with formation of germinal centers a common occurrence (15, 25). Patients with Hashimoto's thyroiditis may be hypothyroid, hyperthyroid, or euthyroid as measured by T_4 levels. This variable level of circulating T_4 is probably a result of hyperactivity of the remaining intact follicular epithelial cells (25). The T_4 levels (euthyroid to hypothyroid) seen with subgroup C avian leukosis virus infection in combination with obvious thyroiditis appear similar to the findings with Hashimoto's thyroiditis.

Insulin levels in the subgroup C virus-infected chickens were significantly elevated by 45 dph. This appears to confirm the involvement of the pancreas which was seen in the histological examination. Insulin plays a key role in lipid metabolism (17), and hyperinsulinemia is associated with hyperlipidemia and obesity (3, 28).

Stunting was significant in all subgroup C virus-infected chickens. The livers from virus-infected chickens were enlarged and resulted in a significantly increased relative liver weight. The cholesterol levels in serum showed a general elevation as the syndrome progressed, but triglyceride levels in serum did not follow the same pattern of elevation (Fig. 1). It appears that all subgroup C avian leukosis viruses resulted in lymphoblastoid infiltration of both the thyroid and the pancreas, but RAV-7 was more efficient in the production of profound hyperlipidemia. A comparison of the subgroup C avian leukosis viruses shows that RAV-7 shares the least antigenic cross-reactivity within the subgroup (12). There could be a correlation between this lack of antigenic relatedness and the increased pathogenicity of RAV-7, but this possibility has not been examined.

OS chickens are the result of inbreeding (8), and their obesity is the phenotypic expression of a genetically determined autoimmune thyroiditis that appears to be the result of changes in at least three separate genetic loci (38). A direct comparison of RAV-7-infected chickens with OS chickens has shown some similarities and differences in the two syndromes. Both syndromes resulted in thyroid infiltration by lymphoblastoid cells, but the RAV-7-infected chickens were affected earlier after hatching (Table 1), and the infiltrating cell appeared to be more immature with RAV-7 infection. There was no evidence of pancreatic involvement in the OS chickens by either histological or hormonal determinations, nor was there a profound hyperlipidemia. We were unable to detect precipitating antithyroglobulin in the sera from any of the RAV-7-infected chickens which were examined, whereas sera from the OS chickens had relatively high titers of precipitating antithyroglobulin. When OS sera were analyzed for the presence of avian leukosis viruses by means of fluorescent-antibody assay of chicken embryo fibroblasts exposed to the OS sera, 1 of 15 samples was found to be positive for the presence of virus antigen p27. This incidence of avian leukosis virus is consistent with the open-flock conditions under which the OS breeding colony was maintained at Cornell University. It appears that the RAV-7 and OS syndromes are not similar outside the thyroiditis and resultant hypothyroidism and that the OS syndrome is probably not the result of avian leukosis virus infection.

The results from the subgroup C avian leukosis virus study provided a basis to examine subgroup specificity of this disease. It appeared that the subgroup C avian leukosis viruses induced a syndrome which was characterized by stunting and lymphoblastoid infiltration of the thyroid and pancreas with resultant hypothyroidism and hyperinsulinemia. In addition to RAV-7, the viruses which were examined were from four other subgroups (A, B, D, and F), some of which had been previously characterized for disease induction. MAV-1(O) (subgroup A) and MAV-2(O) (subgroup B) induce a preponderance of osteopetrosis (30), MAV-2(N) (subgroup B) induces primarily nephroblastoma (37), and RPV (subgroup F) induces rapid appearance of lung angiosarcomas (6). The remaining viruses examined induce a

variety of diseases, predominately lymphoid leukosis, usually after a long latent period (24).

The results of examining eight viruses from four subgroups showed that none of the viruses induced a disease similar to that induced by the subgroup C avian leukosis viruses. Histological examination of the thyroid, liver, and pancreas did not reveal a response typical for subgroup C avian leukosis viruses in any other subgroup (Table 4). At 35 dph, thyroids from subgroup C-infected chickens were uniformly infiltrated by lymphoblastoid cells, with multiple germinal center formation. With the exception of the two subgroup F viruses, there was only a slight alteration in the thyroids from chickens infected with any other subgroup of avian leukosis virus examined. Although both RPV- and RAV-61-infected chickens showed lymphoid infiltration in the thyroids and moderate involvement of the pancreata, the infiltration was focal and appeared secondary to severe lung lesions. Infiltration of the pancreas was present in all chickens except controls and those infected with RAV-7 and may reflect a stress response or a cell which is a common target for avian leukosis viruses. Infection of the pancreas by avian leukosis viruses has been reported, and the infections are generally nonpathogenic (10, 11, 21, 39). Fat accumulation both as adipose and in hepatocytes was apparent only in RAV-7-infected chickens.

A surprising occurrence was the appearance of lymphosarcomas in the livers of the MAV-1(O)-, RAV-50-, and RAV-1-infected chickens. At necropsy, two of the RAV-1-infected chickens had discernible lesions, and microscopic lesions were found in others. The presence of liver tumors with both subgroup A viruses examined presents an interesting question about the disease specificity of this subgroup.

A dramatic increase in insulin levels was found only in RAV-7-infected chickens. Chickens infected with two other viruses (MAV-1(O) and RAV-1) showed significant, but comparatively small, increases in insulin levels, and RAV-50-infected chickens showed a slight increase. Chickens infected with MAV-1(O) and RAV-50 also had more extensive infiltration in the pancreata, whereas RAV-1-infected chickens had only slight infiltration of the pancreas. It was interesting that chickens infected with these three viruses showed pronounced anemia and had liver lymphosarcomas. The liver is a primary receptor for insulin (20), and a reduction in receptors might explain the increased insulin levels.

Decreased levels of T_4 were found in all virus-infected chickens, with significant decreases in those infected with RAV-7, RPV, and RAV-61. Chickens infected with these three viruses all had a degree of thyroiditis. Decreases in T_4 correlated with stunting except for MAV-2(N) (Fig. 2). Thyroid function includes the regulation of growth and metabolism (3, 4, 9, 13), and T_4 regulates the expression of the growth hormone gene (14). Harvey (19) reports an inhibition of growth hormone release in chickens with increased T_4 levels. The correlation of decreased T_4 levels and decreased body weight found in virus-infected chickens appeared to reflect a mechanism other than reduced growth hormone levels, which would result in stunting.

An increase in lipids in serum was apparent only in RAV-7-infected chickens. The triglyceride level was increased to 1,560 mg/dl, whereas the other viruses resulted in no levels above 90 mg/dl. Cholesterol levels followed a pattern similar to that seen for triglycerides. Hyperlipidemia was present in only the RAV-7-infected chickens.

The total syndrome seen in subgroup C avian leukosis virus infection—infiltration of the thyroid and pancreas as well as hypothyroidism and hyperinsulinemia—did not result from infection with subgroup A, B, D, or F avian leukosis viruses. Although chickens infected with subgroup F viruses had a degree of thyroid and pancreas infiltration and a decrease in T_4 levels, no hyperinsulinemia, hyperlipidemia, or fat accumulation resulted. Furthermore, several chickens infected with RPV or RAV-61 had advanced lung lesions which would have been fatal.

The results of this study indicate that the envelope of avian leukosis virus is involved in pathogenic disease. This result provides a basis for further research into the role of the envelope in infection and debilitative disease induced by avian leukosis viruses.

ACKNOWLEDGMENTS

We thank Ted Wheeler and Cheryl Harward for diligent technical assistance, James Proctor of Experimental Pathology Laboratories, Inc., for assistance with histological specimens, and Dean Barnett for consultation. Fertile eggs from OS chickens were obtained through the cooperation of Rod Dietert, and Randy Cole provided helpful comments as the work progressed.

This research was supported by Public Health Service grants CA12323 and CA14236 from the National Cancer Institute. J.K.C. was a predoctoral trainee supported by Public Health Service grant GM07184 from the National Institutes of Health.

LITERATURE CITED

1. **Baluda, M. A., and P. P. Jamieson.** 1961. *In vivo* infectivity studies with avian myeloblastosis virus. Virology **14**:33–45.
2. **Biggs, P. M., B. S. Milne, T. Graf, and H. Bauer.** 1973. Oncogenicity of non-transforming mutants of avian sarcoma viruses. J. Gen. Virol. **18**:399–403.
3. **Bray, G. A., and H. S. Jacobs.** 1974. Thyroid activity and other endocrine glands, p. 413–434. *In* M. A. Greer and D. H. Solomon (ed.), Handbook of physiology, Section 7: endocrinology, vol. 3. American Physiological Society, Washington, D.C.
4. **Bray, G. A., and D. A. York.** 1971. Genetically transmitted obesity in rodents. Physiol. Rev. **51**:598–646.
5. **Carter, J. K., C. L. Ow, and R. E. Smith.** 1983. Rous-associated virus number 7 induces a syndrome in chickens characterized by stunting and obesity. Infect. Immun. **39**:410–422.
6. **Carter, J. K., S. J. Proctor, and R. E. Smith.** 1983. Induction of angiosarcomas by ring-necked pheasant virus. Infect. Immun. **40**:310–319.
7. **Carter, J. K., and R. E. Smith.** 1983. Rapid induction of hypothyroidism by an avian leukosis virus. Infect. Immun. **40**:795–805.
8. **Cole, R. K.** 1966. Hereditary hypothyroidism in the domestic fowl. Genetics **53**:1021–1033.
9. **De Visscher, M., and Y. Ingenblek.** 1980. Hypothyroidism, p. 377–412. *In* M. De Visscher (ed.), The thyroid gland. Raven Press, New York.
10. **DiStefano, H. S., and R. M. Dougherty.** 1969. Multiplication of avian leukosis virus in endocrine organs of congenitally infected chickens. J. Natl. Cancer Inst. **42**:147–154.
11. **Dougherty, R. M., and H. S. DiStefano.** 1967. Sites of avian leukosis virus multiplication in congenitally infected chickens. Cancer Res. **27**:322–332.
12. **Duff, R. G., and P. K. Vogt.** 1969. Characteristics of two new avian tumor virus subgroups. Virology **39**:18–30.
13. **Duran-Garcia, S., J. Gomez-Nieto, M. Fouchereau-Peron, V. F. Padron, M. J. Obregon, G. Morreale de Escobar, and F. Escobar del Rey.** 1979. Effects of thyroid hormones on liver binding sites for human growth hormone, as studied in the rat. Clin. Endocrinol. **11**:275–289.
14. **Evans, R. M., N. C. Birnberg, and M. G. Rosenfeld.** 1982. Glucocorticoid and thyroid hormone transcriptionally regulate growth hormone gene expression. Proc. Natl. Acad. Sci. U.S.A. **79**:7659–7663.
15. **Fisher, D. A., and G. N. Beall.** 1979. Hashimoto's thyroiditis, p.

487–501. *In* J. M. Hershman and G. A. Bray (ed.), The thyroid: physiology and treatment of disease. Pergamon Press, Inc., Elmsford, N.Y.
16. **Fujita, D. J., Y. C. Chen, R. R. Friis, and P. K. Vogt.** 1974. RNA tumor viruses of pheasants: characterization of avian leukosis subgroups F and G. Virology **60**:558–571.
17. **Griminger, P.** 1976. Lipid metabolism, p. 253–262. *In* P. D. Sturkie (ed.), Avian physiology, 3rd ed. Springer-Verlag New York, Inc., New York.
18. **Hanafusa, T., and H. Hanafusa.** 1973. Isolation of leukosis-type virus from pheasant embryo cells: possible presence of viral genes in cells. Virology **51**:247–251.
19. **Harvey, S.** 1983. Thyroid hormones inhibit growth hormone secretion in domestic fowl (*Gallus domesticus*). J. Endocrinol. **96**:329–334.
20. **Hazelwood, R. L.** 1976. Carbohydrate metabolism, p. 211–232. *In* P. D. Sturkie (ed.), Avian physiology, 3rd ed. Springer-Verlag New York, Inc., New York.
21. **Heine, U., G. de The, D. Beard, and J. W. Beard.** 1963. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. V. Elaboration of virus by pancreas of chickens inoculated with the agent. J. Natl. Cancer Inst. **30**:817–835.
22. **Ishizaki, R., and P. K. Vogt.** 1966. Immunological relationships among envelope proteins of avian tumor viruses. Virology **30**:375–387.
23. **Paterson, R. W., and R. E. Smith.** 1978. Characterization of anemia induced by avian osteopetrosis virus. Infect. Immun. **22**:891–900.
24. **Purchase, H. G., W. Okazaki, P. K. Vogt, H. Hanafusa, B. R. Burmester, and L. B. Crittenden.** 1977. Oncogenicity of avian leukosis viruses of different subgroups and of mutants of sarcoma viruses. Infect. Immun. **15**:423–428.
25. **Robbins, S. L., and R. S. Cotran.** 1979. Pathologic basis of disease, p. 1359–1361. The W. B. Saunders Co., Philadelphia.
26. **Robinson, H. L., B. M. Blais, P. N. Tsichlis, and J. M. Coffin.** 1982. At least two regions of the viral genome determine the oncogenic potential of avian leukosis viruses. Proc. Natl. Acad. Sci. U.S.A. **79**:1225–1229.
27. **Robinson, H. L., M. N. Pearson, D. W. DeSimone, P. N. Tsichlis, and J. M. Coffin.** 1979. Subgroup E avian-leukosis-virus-associated disease in chickens. Cold Spring Harbor Symp. Quant. Biol. **44**:1133–1142.
28. **Salans, L. B.** 1981. The obesities, p. 891–916. *In* P. Felig, J. D. Baxter, A. E. Broadus, and L. A. Frohman (ed.), Endocrinology and metabolism. McGraw-Hill Book Co., New York.
29. **Schmidt, E. V., J. D. Keene, M. Linial, and R. E. Smith.** 1982. Association of 3′-terminal RNA sequences with avian leukosis viruses causing a high incidence of osteopetrosis. Virology **116**:163–180.
30. **Smith, R. E., and C. Moscovici.** 1969. The oncogenic effects of nontransforming viruses from avian myeloblastosis virus. Cancer Res. **29**:1356–1366.
31. **Smith, R. E., and E. V. Schmidt.** 1982. Induction of anemia by avian leukosis viruses of five subgroups. Virology **117**:516–518.
32. **Smith, R. E., and L. J. Van Eldik.** 1978. Characterization of the immunosuppression accompanying virus-induced avian osteopetrosis. Infect. Immun. **22**:452–461.
33. **Stone, M. P., R. E. Smith, and W. J. Joklik.** 1974. 35S *a* and *b* subunits of avian RNA tumor virus strains cloned and passaged in chick and duck cells. Cold Spring Harbor Symp. Quant. Biol. **39**:859–868.
34. **Tsichlis, P. N., and J. M. Coffin.** 1979. Role of the *c* region in relative growth rates of endogenous and exogenous avian oncoviruses. Cold Spring Harbor Symp. Quant. Biol. **44**:1123–1132.
35. **Tsichlis, P. N., K. F. Conklin, and J. M. Coffin.** 1980. Mutant and recombinant avian retroviruses with extended host range. Proc. Natl. Acad. Sci. U.S.A. **77**:536–540.
36. **Vogt, P. K.** 1977. The genetics of RNA tumor virus, p. 341–455. Comprehensive virology, vol. 9. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Plenum Publishing Corp., New York.
37. **Watts, S. L., and R. E. Smith.** 1980. Pathology of chickens infected with avian nephroblastoma virus MAV-2(N). Infect. Immun. **27**:501–512.
38. **Wick, G., R. Boyd, K. Hala, L. de Carvalho, R. Kofler, P. U. Muller, and R. K. Cole.** 1981. The obese strain (OS) of chickens with spontaneous autoimmune thyroiditis. Review of recent data. Curr. Top. Microbiol. Immunol. **91**:109–128.
39. **Zeigel, R. F., A. L. Barron, J. H. Kite, and E. Witebsky.** 1970. Virological investigations of chickens with spontaneous autoimmune thyroiditis. II. Examination of tissues by electron microscopy. Avian Dis. **14**:617–619.