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Rapid Induction of Hypothyroidism by an Avian Leukosis Virus

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Infection of 10-day chicken embryos with an avian leukosis virus, RAV-7, resulted in hypothyroidism within 3 weeks posthatching. Histological examination of the thyroids from infected chickens showed an extensive infiltration of lymphoblastoid cells by 7 days posthatching. Areas resembling germinal centers were present in the thyroids of infected chickens by 3 weeks posthatching. Examination of circulating thyroid and pancreas hormones showed a significant reduction in T_3 and T_4 levels and a trend toward higher insulin levels after 16 days posthatching. T_4 supplementation of RAV-7-infected chickens alleviated some aspects of the disease syndrome but did not abrogate all symptoms. Marked involution of both bursa and thymus glands was noted. RAV-7 had an RNA genome of 8.2 kilobases and a polypeptide composition characteristic of an avian leukosis virus. The hypothyroidism followed a dose response to RAV-7 infection.

Avian retroviruses have long been used to provide model systems for tumor formation. Induction of tumors in chickens by these viruses is either an acute response induced by viruses which carry their own oncogene, such as sarcoma viruses or defective leukemia viruses, or a chronic response induced by replication-competent viruses which carry no detectable oncogene (14). The latter group of viruses, the avian leukosis viruses, induce frankly neoplastic growths, such as B-cell lymphomas (4), proliferative disorders, such as osteopetrosis (30), and chronic degenerative diseases, such as anemia (25) and immunosuppression (34). Recent attention has been focused on the chronic debilitative diseases, since they may represent hitherto unrecognized consequences of retrovirus infection and provide model systems for diseases of humans (35).

Chronic debilitation and stunting in animals can be the result of metabolic or endocrine dysfunction, one example of which is obesity and hyperlipidemia in chickens. One model which has received considerable attention is spontaneous autoimmune thyroiditis in chickens (8, 37). In this model, obesity and lipemia are due to hypothyroidism which is caused by lymphoid infiltration of the thyroid gland (38). A second metabolic disease in the chicken is the fatty liver and kidney syndrome observed in broiler chickens at 10 to 30 days of age (20). This disorder is characterized by fat deposition in the liver and kidney, hypoglycemia, reduced hepatic glycogenesis, and reduced hepatic glycogen (3, 7). Fatty liver and kidney syndrome is believed to be associated with biotin deficiency (3), and some features of the syndrome are induced in older chickens by aflatoxin (17). A third disorder, which remains uncharacterized, is the induction of lipemia in chicken embryos by Japanese encephalitis virus (16). In this disease, Japanese encephalitis virus appears to suppress lipid metabolic enzymes, and the circulating lipid reflects the high lipid content of the yolk (16).

We have discovered that Rous-associated virus 7 (RAV-7) causes a syndrome characterized by hyperlipidemia and obesity in chickens (8). When RAV-7 is injected into 10-day-old embryos, chickens show a frank lipemia and fatty liver by 21 days posthatching. The obese and stunted syndrome occurs at high frequency and with high mortality. Chickens which survive an initial high mortality exhibit a low incidence of lymphoid leukosis, osteopetrosis, and nephroblastoma (8). Only one previous report of RAV-7 disease induction has been published (17), and RAV-7 is reported to cause a low incidence of lymphoid leukosis and other neoplasias when injected into hatched chickens (17).

The purpose of the present study was to examine the role of the thyroid and pancreas in the RAV-7-induced disease syndrome. We examined histological sections of the thyroid and pancreas and levels of circulating thyroid and pancreas hormones of RAV-7-infected chickens

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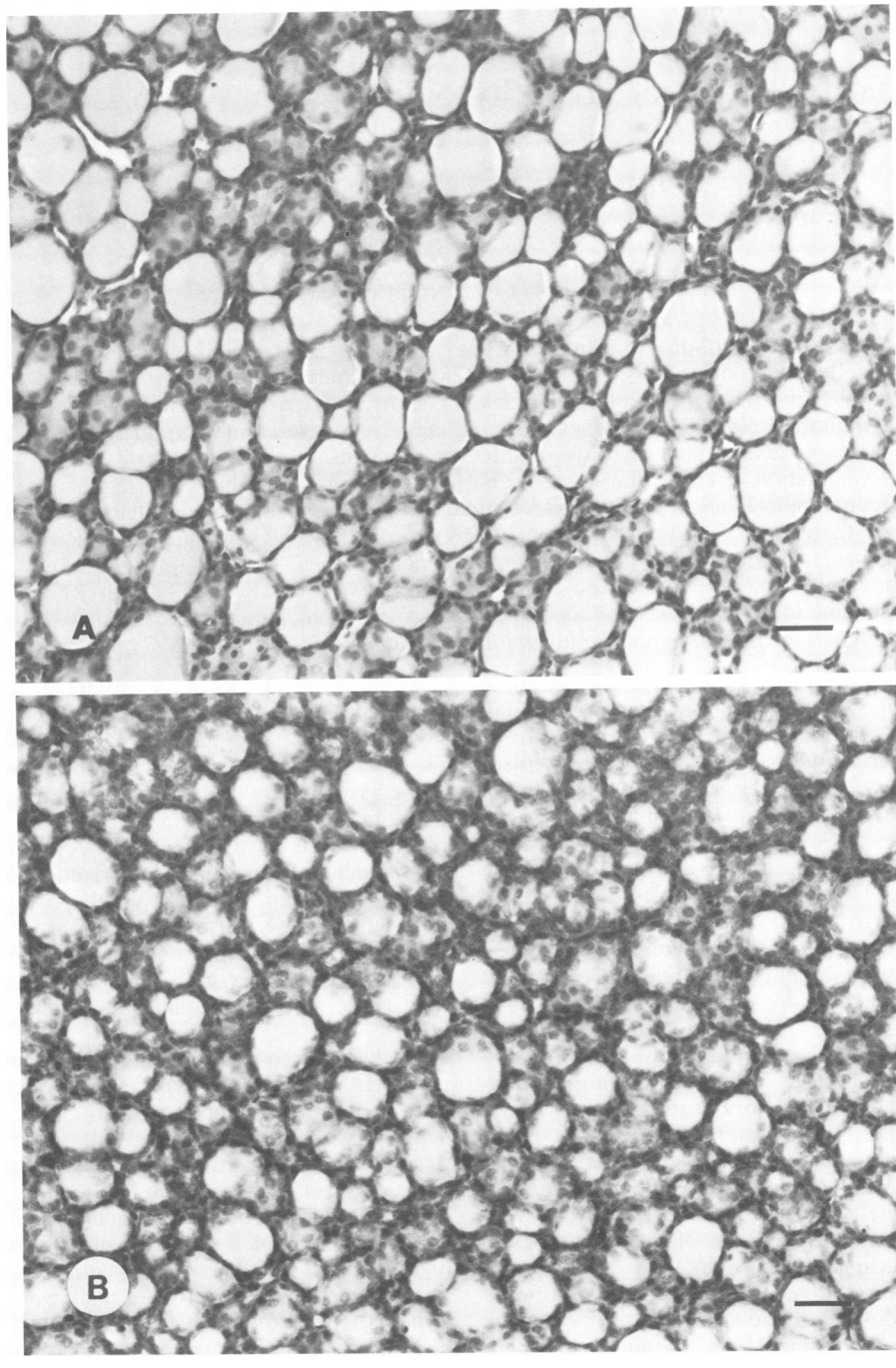
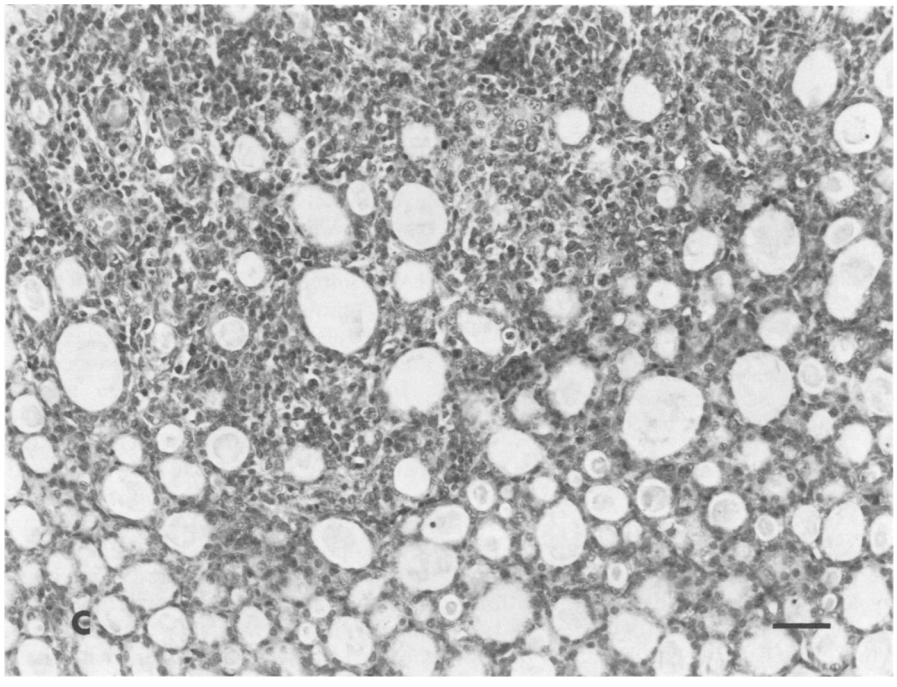


FIG. 1. Histological sections of thyroid glands from RAV-7-infected and uninfected chickens. Thyroid glands were treated as described in the text. All sections were stained with hematoxylin and eosin. Bar, 30 μm. (A) Thyroid from an uninfected chicken 2 days posthatching. The follicular epithelial cells are squamous, and the colloid appears uniform. (B) Thyroid tissue from a RAV-7-infected chicken 2 days posthatching. The follicular epithelial cells, in some areas, show an increased height, and the colloid is pale and not uniform in appearance. (C) Thyroid tissue from a RAV-7-infected chicken 7 days posthatching. The follicular epithelial cells are columnar and contain numerous large vacuoles. The colloid is not uniform in appearance. Lymphoblastoid cells with large nuclei and prominent nucleoli are infiltrating the tissue replacing the normal thyroid structure.



and uninfected chickens during the development of the disease syndrome. We show that RAV-7 caused endocrine changes which accounted for the hyperlipidemia and stunting observed in infected chickens.

MATERIALS AND METHODS

Abbreviations. RAV-7, Rous-associated virus 7; MAV-2(0), myeloblastosis-associated virus of subgroup B inducing predominantly osteopetrosis; SAT, spontaneous autoimmune thyroiditis; OS, obese strain of chickens from the Cornell C strain; T_3, triiodothyroxine; T_4, thyroxine.

Virus. RAV-7 was initially isolated from a stock of Bryan high-titer Rous sarcoma virus (14). The RAV-7 used for this study was obtained from C. Moscovici, Veterans Administration Hospital, Gainesville, Fla., and was grown in chicken embryo fibroblasts. The virus used in this study was biologically purified by two rounds of end-point purification on C/O chicken embryo fibroblasts and was stored at −70°C. RNA sizing and protein electrophoresis procedures were previously reported (33).

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) with 10^4 infectious units of RAV-7. After infection as embryos, chickens hatched with an efficiency of about 90%. Infected chickens were hatched and reared in an isolation room of the Animal Laboratory Isolation Facility of the Duke University Comprehensive Cancer Center. Uninfected chickens were hatched and reared in a separate room of the same facility.

Histological examination. At selected times during the course of the disease, chickens were sacrificed by intravenous administration of pentobarbital. Tissue specimens were collected immediately and fixed in 10% buffered formalin, dehydrated, embedded, sectioned, and stained with hematoxylin and eosin or methyl green pyronin, following conventional procedures. Tissue specimens were collected from uninfected hatchmates of RAV-7-infected chickens and were processed identically. All histological sections were prepared by Experimental Pathology Laboratories, Inc., Raleigh, N.C.

Hormone assays. Circulating T_3, T_4, insulin, and glucagon levels were determined, using radioimmunoassay kits (Cambridge Medical Diagnostics, Inc., Billerica, Mass.). Samples were collected following the protocols provided with the kits. All chickens were fasted overnight before sample collection. Blood samples for glucagon assays were collected by jugular venipuncture into sodium-EDTA (14 mg/10 ml of blood). The samples were immediately placed on ice, and the protease inhibitor aprotinin was added at 1 mg/10 ml of blood; samples remained on ice until plasma was separated by centrifuging at 2,000 rpm for 10 min. An ultracentrifugation step of 70,000 × g for 60 min was performed on all samples to remove lipid, since many samples were visibly lipemic. Samples not assayed on the day of collection were frozen and stored at −20°C. Assays for chickens 2 weeks post-hatching or younger were run on pooled samples of equal volume from 2 to 3 chickens. All samples were run in duplicate. Samples from uninfected age-matched controls were collected, processed, and stored identically and were run simultaneously with samples from infected chickens.

T_4 supplementation. Age-matched chickens, either infected with RAV-7 as 10-day-old embryos or uninfected, were separated into four groups. The groups

were divided as follows: group 1, eight uninfected chickens which received saline; group 2, six uninfected chickens which received T_4; group 3, eight RAV-7-infected chickens which received saline; and group 4, five RAV-7-infected chickens which received T_4. Chickens were given either 4 μg of Synthroid (levothyroxine sodium; Flint Laboratories, Deerfield, Ill.) dissolved in 0.9% NaCl (pH 10) per 100 g of body weight or an equivalent volume of 0.9% NaCl (pH 10) on a daily basis via subcutaneous injection. The chickens were weighed weekly, and dosage was modified to adjust for weight gain. At 31 days posthatching, all chickens were sacrificed, serum samples were taken to determine circulating hormone levels, and body and liver weights were taken and tissue samples were obtained for histological examination.

Clinical chemistry. Serum triglycerides and cholesterol levels were determined by procedures previously reported (8).

RESULTS

Histology. Preliminary evidence for thyroid involvement indicated a need to examine the sequence and extent of thyroid alteration. Therefore, the first experiment was designed to examine thyroid tissue at various times during RAV-7 infection. Thyroids of RAV-7-infected chickens taken at 2 days posthatching showed slight alteration, with the appearance of increased activity of the follicular epithelial cells (Fig. 1). The follicular colloid in the thyroid of RAV-7-infected chickens at 2 days posthatching (Fig. 1B) did not appear as uniform as the follicular colloid in the thyroids from uninfected hatchmates (Fig. 1A). By 7 days posthatching, approximately 50% of the normal thyroid tissue was replaced by lymphoblastoid cells in RAV-7-infected chickens (Fig. 1C). As the disease syndrome progressed, the infiltration obliterated most of the normal thyroid tissue. Areas resembling germinal centers were noted by 3 weeks posthatching, and by 5 weeks posthatching, germinal centers were prominent (Fig. 2B). Follicular epithelial cells late in infection were columnar to cuboidal, which indicated increased activity in these cells. Cells infiltrating the thyroid appeared to be immature members of the lymphoid series. The nuclei of the infiltrating cells were large, with prominent nucleoli, and cytoplasms were slightly eosinophilic. Few plasma cells were noted. At times, the thyroid-thymus barrier was indistinct. Lymphoblastoid cells of similar morphology were observed to infiltrate the pancreases of RAV-7-infected chickens, but the infiltration was localized, and normal pancreatic tissue remained throughout the disease process.

Hormone assays. The morphological evidence presented above suggested that the function of the thyroid and pancreas might be altered. To establish whether a functional alteration was present in these organs, we determined circulating levels of T_4, T_3, insulin, and glucagon on 19 pairs of RAV-7-infected and uninfected hatchmates over a 43-day period. The most dramatic effect was on the level of T_4 (Fig. 3). During the first 2 weeks, no difference was noted in T_4 levels, but subsequent samples showed that T_4 levels in RAV-7-infected chickens were significantly lower than in uninfected chickens ($P < 0.001$); by 40 days posthatching, the T_4 level in a RAV-7-infected chicken was 19% that of a normal hatchmate (Fig. 3). The levels of circulating T_3 were also decreased after 14 days (Fig. 4), but the decrease was not as pronounced ($P < 0.01$).

Insulin levels were above normal levels through 43 days posthatching, and ranged from 114 to 352% of normal. Exceptions to the high insulin levels were the 16-, 30-, and 37-day samples, which were below normal. Glucagon levels remained close to normal, except at 20 to 30 days posthatching when the levels dropped to 34 to 44% of normal (Table 1). It is interesting to note that 20 to 30 days after hatching was the time of highest mortality (data not shown). Although trends were apparent with the glucagon and insulin levels, the values were not significantly different for the RAV-7-infected chickens. By 8 days posthatching, RAV-7-infected chickens were stunted, as indicated by a body weight 78% of normal. The body weights remained below normal and were 34.7% of normal at 43 days posthatching (Table 1).

T_4 supplementation. To examine the role of hypothyroidism in the RAV-7 disease syndrome, the effect of T_4 supplementation was examined. The results of this study are presented in Table 2. T_4 supplementation led to increased body weight, decreased liver weight expressed as percentage of body weight, increased T_3 and T_4 levels, and decreased insulin levels in T_4-treated RAV-7-infected chickens (group 4) versus saline-treated RAV-7-infected chickens (group 3). Group 3 showed highly significant differences ($P < 0.01$) in body weight, liver weight expressed as percentage of body weight, and T_3 and T_4 values when compared with the saline-treated uninfected chickens (group 1). All parameters except body weight and liver weight expressed as percentage of body weight showed a shift toward normal, which was reflected by a reduced level of significance between groups 1 and 4 (Table 2). Body weight, liver weight expressed as percentage of body weight, and T_4, insulin, and glucagon levels were significantly different ($P < 0.05$) between the two RAV-7 groups, indicating a shift toward normal with T_4 supplementation (Table 2). The uninfected chickens which received T_4 (group 2) had reduced T_3 and T_4 levels. Glucagon levels were decreased with T_4 supplementa-

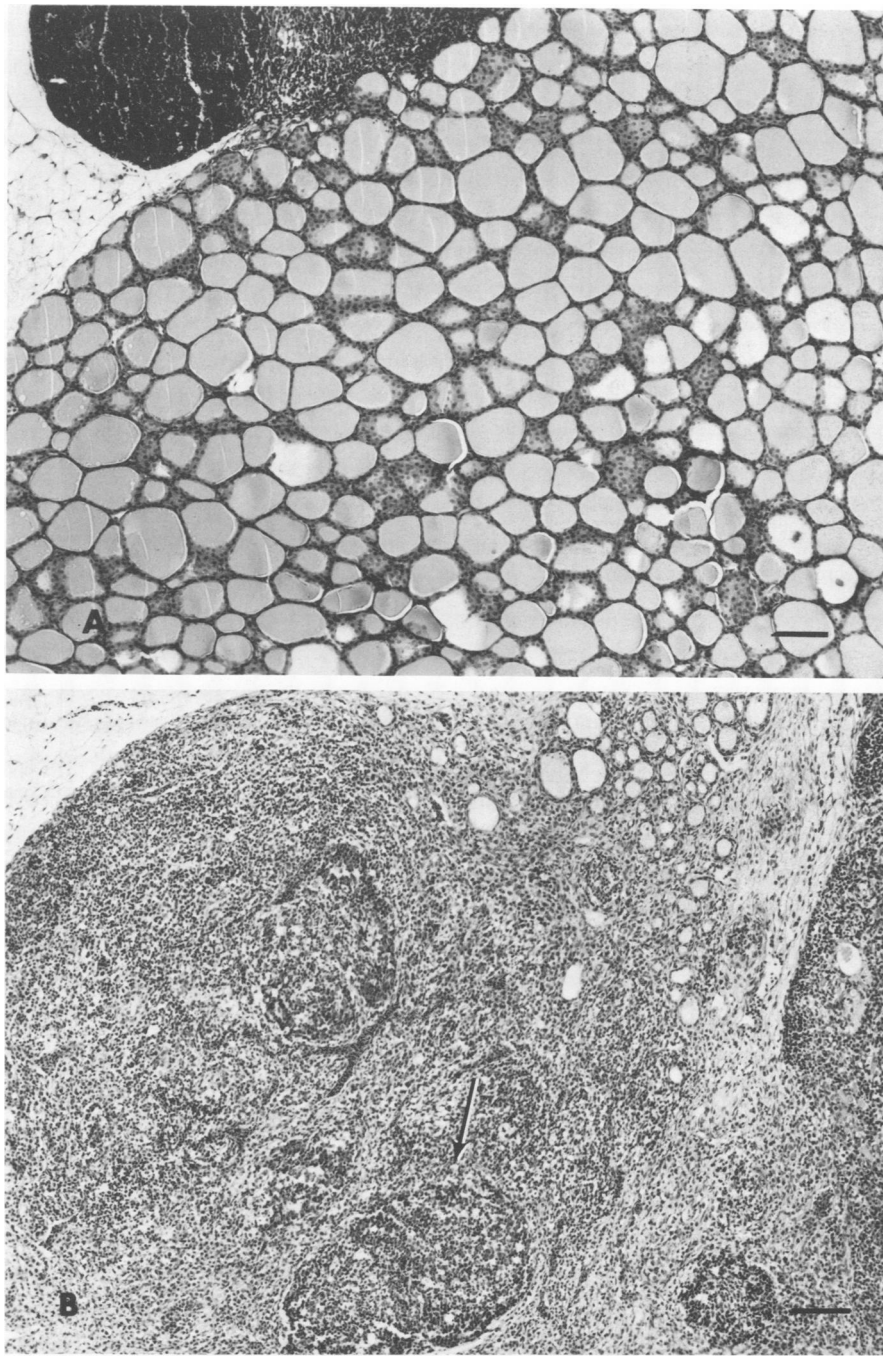


FIG. 2. Histological sections of thyroid glands from RAV-7-infected and uninfected chickens at 5 weeks posthatching. Thyroids were treated as described in the legend to Fig. 1. Bar, 75 μm. (A) Thyroid tissue from an uninfected chicken. The follicles are well defined, with uniform colloid and squamous epithelial cells. (B) Thyroid tissue from a RAV-7-infected chicken. The organ is extensively infiltrated by lymphoblastoid cells, and the formation of areas resembling germinal centers is apparent (arrows). The follicles which remain intact appear to be normal and highly active.

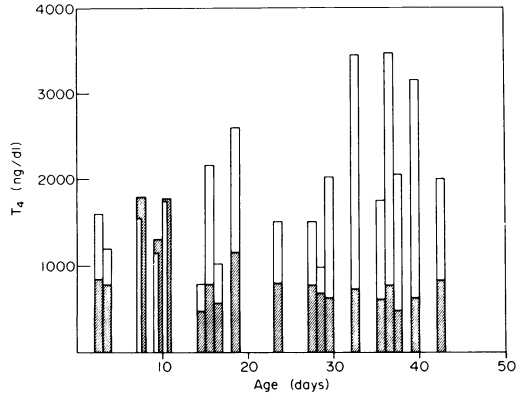


FIG. 3. Levels of T_4 in RAV-7-infected and uninfected chickens. Hormone assays were performed as described in the text on infected and uninfected chickens of the same age during a 43-day observation period. Values for RAV-7-infected chickens are shown by shaded bars, whereas values for uninfected chickens are shown by open bars. Each determination was performed in duplicate, and each bar represents an infected-uninfected chicken comparison.

tion regardless of whether the group of chickens was infected with RAV-7.

Virus. Rapid onset of disease induced by avian retroviruses is usually associated with sarcoma or defective viruses which have an RNA size of 9.7 kilobases or much smaller than 8.2 kilobases, respectively. To establish that RAV-7 is a leukosis virus, RNA sizing and protein characterization of the virus was performed. RAV-7 had an 8.2-kilobase RNA, as

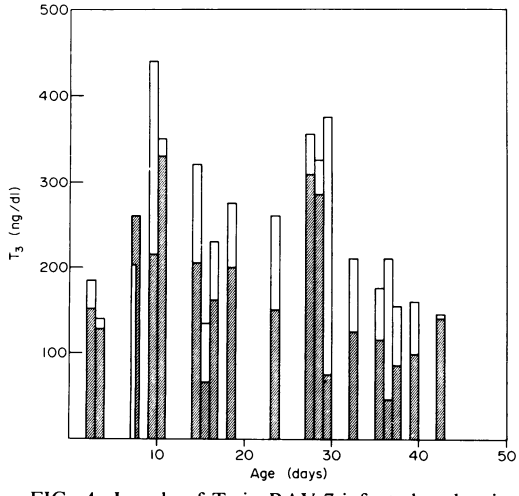


FIG. 4. Levels of T_3 in RAV-7-infected and uninfected chickens. See the legend of Fig. 3 for symbol explanation.

TABLE 1. Circulating hormone levels in RAV-7-infected chickens[a]

Age (days)	Virus	Wt (g)	Insulin (μU/ml)	Glucagon (ng/ml)
3	−	34	6	ND[b]
3	+	31 (91.2)[c]	6 (100)	ND
8	−	59	ND	3,800
8	+	46 (78.0)	ND	4,000 (105.3)
10	−	ND	8.1	1,950
10	+	ND	7.1 (87.7)	4,000 (205.1)
11	−	50	6	ND
11	+	39 (78.0)	6 (100)	ND
16	−	78	7	2,750
16	+	73 (93.6)	17.5 (250)	2,000 (72.7)
17	−	101	7.6	2,900
17	+	80 (79.2)	6.4 (74.4)	3,550 (122.4)
19	−	107	6	2,150
19	+	88 (82.2)	9.5 (158.3)	2,200 (102.3)
24	−	ND	6	4,000
24	+	ND	7.4 (123.3)	1,750 (43.8)
28	−	248	7.8	3,100
28	+	168 (67.8)	17 (217.9)	1,050 (33.9)
29	−	233	6	3,900
29	+	113 (48.5)	9.8 (163.3)	3,900 (100)
30	−	256	13	3,450
30	+	149 (58.2)	9.8 (75.4)	1,275 (37.0)
33	−	250	9.4	3,300
33	+	157 (62.8)	12.5 (133.0)	3,000 (90.9)
36	−	303	9.6	1,550
36	+	135 (44.6)	11.5 (119.8)	1,580 (101.9)
37	−	340	19.5	2,375
37	+	110 (32.4)	7 (35.9)	1,675 (70.5)
38	−	ND	6.4	3,300
38	+	ND	22.5 (351.6)	4,000 (121.2)
40	−	343	13.5	ND
40	+	189 (55.1)	6 (143.6)	ND
43	−	577	6	4,000
43	+	200 (34.7)	14.5 (241.7)	3,700 (92.5)

[a] Samples were taken according to the protocol provided by Cambridge Medical Diagnostics, Inc., for use with their radioimmunoassay kits. Samples for young birds were pooled from 2 or 3 birds, using equivalent amounts per bird. All samples were ultracentrifuged before assay to remove lipid.

[b] ND, Not determined.

[c] Values expressed as percentage of control value.

determined by agarose gel electrophoresis. The protein banding pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis was typical of avian leukosis virus, and bands corresponding to gp85, gp37, p27, p19, p12, and p15/p10 were observed (data not shown).

Effect of viral dilution. To further establish the role of RAV-7 in the induction of the disease process, we performed an experiment in which chicken embryos were infected with dilutions of the virus, and chickens were sacrificed and examined 39 days after hatching. Viral dilutions of 1:20 to 1:200 had the most pronounced effect, in that the levels of triglycerides and cholesterol were markedly elevated (Fig. 5). Body weights and T_4 levels were decreased at every dilution employed, whereas insulin levels and liver weights expressed as percentage of body weight were elevated at most viral dilutions (Fig. 5). T_3 levels were decreased when higher RAV-7 dilutions (1:200 to 1:200,000) were injected (Fig. 5). These results suggest that RAV-7 interacted with chicken embryos in a dose-dependent manner and that relatively high dilutions of virus (up to 1:200,000) were effective in causing the disease syndrome.

Effect of RAV-7 on lymphoid organs. Avian leukosis viruses of subgroups A and B have been examined for the ability to cause lymphoid involution and immunosuppression, and subgroup B viruses cause lymphoid involution (34) and immunosuppression (29, 34) whereas subgroup A viruses cause neither (26, 29). It was therefore of interest to determine whether RAV-7, a leukosis virus of the C subgroup, caused lymphoid organ involution. It was found that RAV-7 infection caused a marked reduction in the size of the bursa and thymus, whereas the spleen size remained essentially unchanged in 3-week-old RAV-7-infected chickens (Table 3). Examination of the spleens of chickens older than 3 weeks revealed that splenic involution was present from 4 weeks onward (data not shown).

DISCUSSION

Thyroid tissue from chickens 2 days posthatching appeared to show some alteration with RAV-7 infection, but the change was not pronounced. Examination of thyroid tissue from RAV-7-infected chickens revealed lymphoblastoid infiltration as early as 7 days posthatching. Follicular epithelial cells at this time had large vacuoles and increased eosinophilia of the cytoplasm, which was indicative of cellular degeneration. By 16 days posthatching, the T_3 and T_4 levels in RAV-7-infected chickens were significantly below normal, a finding which may reflect the extensive infiltration of the thyroid and lack of normal thyroid structure. Continued low T_3

TABLE 2. T_4 supplementation[a]

Group	T_4	Virus	No. of birds	Body wt (gm)	Liver wt (gm)	Liver wt/body wt (%)	T_3 (ng/dl)	T_4 (ng/dl)	Insulin (μU/ml)	Glucagon (ng/ml)
1	−	−	8	220.5 ± 17.0	5.89 ± 0.72	2.7 ± 0.28	162.2 ± 23.8	1,687.5 ± 47.9	7.4 ± 5.6	3,200 ± 550
2	+	−	6	209.2 ± 14.6[b]	5.57 ± 0.6	2.68 ± 0.17[b]	112.3 ± 12.5	1,175.0 ± 66.1[c]	7.7 ± 2.0	2,300 ± 240[c]
3	−	+	8	131.0 ± 22.6[c]	5.5 ± 1.0	4.2 ± 0.35[c]	77.3 ± 6.8[c]	533.8 ± 25.6[c]	14.4 ± 3.4[d]	3,400 ± 1,080
4	+	+	5	157.4 ± 28.8[c,d]	5.48 ± 1.1	3.5 ± 0.43[c,e]	112.7 ± 25.4	990.0 ± 115.3[b,e]	6.8 ± 0.2[b,e]	2,300 ± 600[b,d]

[a] T_4 (Synthroid) was given at the rate of 4 μg/100 g of body weight. Saline injections of 0.9% NaCl (pH 10) were used as a placebo. Values given are means ± standard deviation and were obtained at necropsy when birds were 31 days posthatching. Statistical analysis was performed by the Student *t* test.

[b] $P < 0.05$ when compared with group 1.

[c] $P < 0.01$ when compared with group 1.

[d] $P < 0.05$ when compared with group 3.

[e] $P < 0.01$ when compared with group 3.

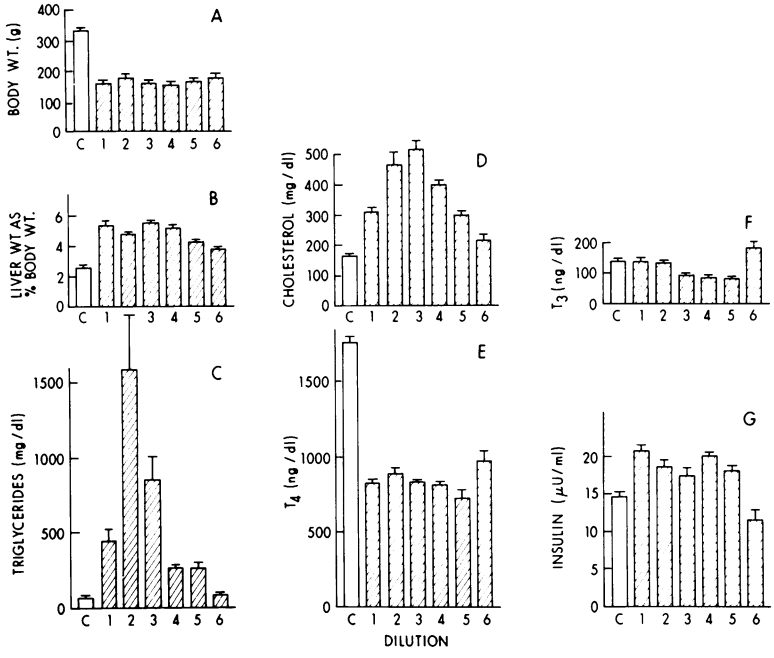


FIG. 5. Dose response to RAV-7. RAV-7 was given to 10-day-old chicken embryos by the intravenous route (0.1 ml of the indicated dilution), and the chickens were hatched and examined at 39 days of age. Values shown are means ± the standard error of the mean. C, Uninfected hatchmates; 1, 1:2 dilution of RAV-7 stock; 2, 1:20 dilution of RAV-7 stock; 3, 1:200 dilution of RAV-7 stock; 4, 1:2,000 dilution of RAV-7 stock; 5, 1:20,000 dilution of RAV-7 stock; 6, 1:200,000 dilution of RAV-7 stock. The original titer of the RAV-7 stock was 10^6 infectious units per ml; the 1:200,000 dilution therefore contained approximately 5 infectious units of virus.

and T_4 levels resulted in increased activity of the remaining follicular epithelial cells, as indicated by columnar-to-cuboidal appearance and increased basophilia of these cells. The changes in thyroid appearance and circulating thyroid hormones occurred before the appearance of hyperlipidemia at approximately 20 days posthatching (8). The apparent hypothyroidism in RAV-7-infected chickens may therefore cause the hyperlipidemia manifested later in the course of the disease.

Hyperlipidemia is a common occurrence in hypothyroidism, an occurrence which can be explained by alterations in the metabolism of lipids and phospholipids (13, 28). Correze and co-workers (12) reported changes in hexokinase I, glucose 6-phosphate dehydrogenase, gluconate 6-phosphate dehydrogenase, and cyclic AMP accumulation in hypothyroidism in rats. These changes result in shunting glucose into fatty acid synthesis. Severson and Fletcher (31) reported a decrease in activity of acid cholesterol ester hydrolase in rat liver and fat pad cells from thyroidectomized rats with a net reduction in cholesterol catabolism. The net result is that the decrease in lipid utilization or catabolism

TABLE 3. Lymphoid organ involution caused by RAV-7

Virus[a]	No. of birds observed	Body wt	Bursa (as % body wt)[b]	Thymus (as % body wt)	Spleen (as % body wt)
−	5	135.4 ± 5.4	0.675 ± 0.030	0.736 ± 0.057	0.159 ± 0.013
+	7	97.4 ± 6.8	0.214 ± 0.125	0.203 ± 0.116	0.178 ± 0.078
Level of significance[c]		$P < 0.001$	$P < 0.001$	$P < 0.001$	NS

[a] RAV-7 was given to 10-day-old embryos by the intravenous route at 10^4 infectious units per embryo. Chickens were sacrificed and examined at 20 days of age.

[b] Lymphoid organ weights are expressed as a percentage of the body weight.

[c] Levels of significance according to Student's two-tailed *t* test. NS, No significant difference.

overshadows the slight decline or unchanged levels in lipid synthesis in hypothyroid animals (19).

There is a striking similarity between RAV-7-infected chickens and OS chickens, both phenotypically and by the presence of hypothyroidism. SAT is a genetically determined characteristic in Cornell C strain chickens which, after inbreeding, led to an obese chicken line (OS) (10, 11). The OS chickens exhibit obesity, high levels of antibodies to thyroglobulin, antibodies to thyroid microsomal components, infiltration of the thyroid by lymphoid cells, and a deficiency in T_3 and T_4 (37, 39). Appearance of obesity, reduced skeletal structure, and long, silky feathers are usual by 3 to 5 weeks posthatching, although a reduction in T_4 levels and an increase in ^{131}I uptake are reported in newly hatched OS chickens (R. Sundick, M. Livezey, T. Brown, and N. Bagchi, Fed. Proc. 35:512, 1976). The basis of SAT is hypothesized to be multigenic and involves a primary thyroid defect and defects in immune regulation (38). Although early work reports lymphoid infiltration of OS thyroids at 1 to 3 weeks posthatching (21), most work emphasizes the appearance of lymphoid and plasma cells at 3 to 5 weeks posthatching (37). The onset of the RAV-7 syndrome and infiltration of the thyroids appeared slightly earlier than that reported for SAT. Although the thyroids of RAV-7-infected chickens were infiltrated by lymphoblastoid cells, there was little evidence for the presence of large numbers of plasma cells characteristic of SAT (37), and the infiltrating cells in RAV-7-infected chicken thyroids appeared to be more immature than those seen in SAT (G. Wick, personal communication). Experiments are in progress to determine the identity of the cells which infiltrate RAV-7-infected thyroids. It will be of fundamental interest to establish whether a relationship exists between the infiltrating cell and the thymus and bursa gland involution which was observed in RAV-7-infected chickens (Table 3). There was no evidence of antithyroglobulin in RAV-7 infection (unpublished observations), and the presence of other autoantibodies has not been determined. These observations appear to distinguish the RAV-7 syndrome from SAT. In work with rats which exhibit spontaneous thyroiditis, Kotani and co-workers (22) report a better correlation between the extent of thyroiditis and the presence of anti-follicular cell antibodies than between thyroiditis and antithyroglobulin. The presence of autoantibodies other than antithyroglobulin in the disease syndrome induced by RAV-7 is an interesting possibility which has not been examined.

Chickens which are hypothyroid and have goiters induced by tapazole exhibit near normal growth characteristics when given daily subcutaneous injections of T_4 at a dosage of 3 to 4 μg/100 g of body weight (32). The early diagnosis of SAT as a hypothyroid condition was made by supplementation of the diet of OS chickens with T_4, which results in abrogation of the syndrome (36). In the present study, supplementation of RAV-7-infected chickens with Synthroid showed some alleviation of disease symptoms. In comparison with the saline-treated RAV-7-infected chickens, T_4-supplemented RAV-7-infected chickens showed a shift in all parameters toward normal. However, T_4 supplementation did not abrogate the disease because significant stunting and alteration of circulating hormone levels were still present when saline-treated uninfected chickens were compared with T_4-treated RAV-7-infected chickens. The decreased T_4 levels in the uninfected chickens which received Synthroid were not surprising because the exogenous hormone could result in a negative feedback on stimulation of in vivo T_4 production by the thyroid. The T_4-treated uninfected chickens exhibited mild thyroid hypoplasia that was apparent upon histological examination of their thyroid glands.

Our attention has been drawn to avian retrovirus debilitation because stunting accompanies several disease syndromes. For example, infection of chickens with an avian osteopetrosis virus, MAV-2(0), results in chickens which grow at a rate 26% of that of uninfected hatchmates (2). In the case of MAV-2(0)-induced stunting, bone cell proliferation probably contributes a substantial tumor load to the infected chicken. However, it is clear that viral plaque isolates which lead to little or no bone proliferation nevertheless cause a decrease in body weight (33). In the case of another avian leukosis virus, ring-necked pheasant virus, a failure of infected chickens to grow accompanies the appearance of lung tumors (9). Work is in progress to look at the subgroup specificity of the RAV-7 syndrome. In the case of both virus infections cited above, it is clear that stunting is most severe when a cellular proliferation is present, but animals with no obvious growths are frequently debilitated. RAV-7 induced significant stunting without evidence of cellular proliferation (Table 1). In previous studies, RAV-7-induced stunting is significant by 14 days posthatching, and growth rates of 3.9 g/day and 16.3 g/day are present in RAV-7-infected- and uninfected chickens, respectively (8).

Hypothyroidism is reported to cause increased insulin release and decreased insulin utilization, which leads to a net increase in insulin levels (5, 6). T_4 is also postulated to function by increasing the numbers of receptors for insulin, glucagon, and β-adrenergic catechol-

amines (23). The increased number of hormone receptors increases the turnover in these compounds and lowers their levels in circulation. The decreased utilization of insulin in hypothyroidism can be the result of decreased receptors for insulin. Localized lymphoblastoid infiltration of the pancreas in RAV-7 infection has been reported (8) and suggests a primary effect of the virus on pancreatic function. A secondary effect on pancreatic hormone levels could be produced by the hypothyroid condition, which could explain the slightly increased insulin levels seen with decreased T_4 levels (Table 1). The reduction of insulin levels with T_4 supplementation would indicate an interaction between the hormones (Table 2). The reduced levels of glucagon at 20 to 30 days posthatching may be an expression of specific infiltration of alpha islets in the chicken pancreas. The avian pancreas contains predominantly islets with alpha cells or with beta cells rather than islets with both alpha and beta cells (18, 24). Some evidence of fibrosis in the pancreases of RAV-7-infected chickens is found with electron microscopy, but the fibrosis has not been localized to a specific islet type (unpublished observation). The transient nature of the glucagon decrease could be a result of resolution of the damage in the pancreas. It is interesting to note that the largest glucagon decrease corresponded with the time of highest mortality. Removal of the splenic lobe of the avian pancreas, which contains a majority of alpha islets, results in hypoglycemia and death (24) and indicates an important role of glucagon in normoglycemia and survival in the chicken.

The evidence presented here indicates that infection of chickens by an avian leukosis virus, RAV-7, induced hypothyroidism. Thyroid alteration was supported by morphological evidence for lymphoblastoid cell infiltration and biochemical evidence for an alteration of circulating T_3 and T_4 levels in RAV-7-infected chickens. Evidence for alteration in pancreatic function was indicated by morphological studies, but the circulating insulin and glucagon levels did not give a clear indication of functional alteration. The mechanism of alteration was infiltration of the endocrine glands by lymphoblastoid cells and, in the case of the thyroid, resultant disappearance of normal tissue. The disease syndrome expressed, stunting and hyperlipidemia, was explained by the hypothyroidism. The identification of the cell infiltrating the thyroid and pancreas, why that cell is homing to these specific targets, and the subgroup specificity of the syndrome remains to be elucidated.

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