Detection and Assay of Avian Tumor Virus Group-Specific Antigen and Antibody by the Paired Radioiodine-Labeled Antibody Technique

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The paired radioiodine-labeled antibody technique (PRILAT) was applied to the detection and quantitation of avian tumor virus group-specific (gs) antigens and antibody. The technique proved to be specific, repeatable, and appreciably more sensitive than the microcomplement-fixation test for avian leukosis (COFAL). The PRILAT facilitated direct measurement of comparative antigen content of several types of transformed, neoplastic, or virus-infected cells and the magnitude of non-specific antibody binding by appropriate control cells. The versatility of the technique was illustrated by application to the detection and quantitation of gs antibody content of chicken, turkey, pigeon, and hamster sera. Antibodies were detected in COFAL-negative sera from hamsters bearing tumors induced by the Schmidt-Ruppin strain of Rous sarcoma virus. Sera from chickens bearing similar tumors were not positive for gs antibodies, although sera from turkeys and chickens immunized with avian leukosis virus did contain gs antibodies.

Antibodies to avian leukosis virus group-specific (gs) antigens are usually demonstrated by direct complement-fixation CF tests (microcomplement-fixation test for avian leukosis, COFAL) in sera of mammals bearing Rous sarcoma virus (RSV)-induced tumors (2, 4-6, 8, 13, 15, 19, 22, 25). Similar tests have demonstrated gs antibodies in sera of immunized pigeons but not in sera from chickens and turkeys, which do not fix guinea pig complement (19, 20). In the present study, the paired radioiodine-labeled antibody technique (PRILAT), as adapted by Yohn and colleagues (10, 16) to adenovirus 12 tumor serology, was employed to measure avian leukosis gs antigen(s) in RSV tumor (1) and transformed cells. Antibodies to this antigenic complex (2, 9, 12) were detected in COFAL-negative sera from hamsters bearing RSV-induced tumors. Chicken and turkey sera known to contain antibodies to avian leukosis virus envelope antigens (9, 14, 21, 24) also apparently contained low levels of gs antibody. A preliminary oral report of this study has been presented (D. S. Yohn, J. Weber, and J. R. McCammon, Proc. Amer. Ass. Cancer Res. 12:22, 1971).

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MATERIALS AND METHODS

Virus. Purified Schmidt-Ruppin strain RSV (SR-RSV-D) was obtained from P. Sarma of the National Institutes of Health. Stocks were prepared by clarifying homogenates of wing-web tumors induced in 2-week-old chicks from a leukosis-free flock (SPAFAS, Norwich, Conn.). The virus titers ranged from 10^5 to 6×10^5 focus-forming units/ml on chick embryo cells (24).

Cell cultures. Three hamster cell lines transformed with RSV in vivo or in vitro were employed in these studies. A Bryan RSV-induced hamster tumor cell line was obtained from E. Fleissner (12). This cell line was originated by P. Sarma (23) and is identified as H-RSV(BH). A twice-cloned SR-RSV transformant of BHK-21 (clone 13) cells was also obtained from E. Fleissner and is identified as BHK-RSV(SR) (12). An SR-RSV-induced hamster tumor cell line designated SRHT, was purchased from Flow Laboratories, as were BHK-21 cells.

Primary chicken embryo fibroblast (CEF) cultures were prepared from 12-day-old embryonated SPAFAS eggs. Chicken tumor cells were grown from primary SR-RSV-D-induced wing-web tumors of 2-week-old SPAFAS chicks.

All cells were grown in Eagle minimal essential medium (MEM) supplemented with 5 to 10% Tryptose phosphate broth and 5 to 10% fetal calf serum. Cells were grown as monolayers prior to seeding on cover slips in Leighton tubes. Cover slips of infected or uninfected cells were rinsed in three changes of

phosphate-buffered saline (PBS), air-dried, and fixed in acetone at -21°C for 10 to 15 min. They were air-dried again and stored at -70°C until used for immunofluorescence or PRILAT.

CF tests. The test used was the COFAL (22). The CF antigen was solubilized in saline by homogenizing SR-RSV-D chicken tumors which had been frozen and thawed three times. Particulate material was removed by centrifugation at $1,000 \times g$. All reactions employed 5 C/H_{50} units of complement and 3 units of antigen. Known positive and negative controls as well as complement controls were included with each test. The sera were inactivated for 15 min at 56°C prior to testing.

Immunofluorescence. Indirect immunofluorescence was performed essentially as described previously (15). The primary reagent consisted of COFAL-positive or normal hamster serum. The secondary reagent was fluorescein-conjugated rhesus monkey anti-hamster immunoglobulin (IgG) which had been absorbed with a washed pellet of frozen-thawed BHK-RSV(SR) cells and with an acetone powder of hamster kidney. Positive, negative, and secondary reagent controls were included in each test.

Antisera. COFAL-positive sera were obtained from Syrian hamsters (inbred PD₄) bearing primary SR-RSV-induced tumors. Hamster sera were titrated individually by CF, and those with a COFAL titer of 1:64 or greater were pooled. Chicken sera were obtained from SPAFAS chickens bearing wing-web tumors induced with SR-RSV. P. Sarma kindly supplied pigeon anti-SR-RSV-B and normal chicken, turkey, and pigeon sera. Chicken antisera, anti-H-RSV (Harris strain) and anti-RAV-2, and turkey antisera, anti-SR-RSV, anti-H-RSV, and anti-559-RSV (strain 559), were supplied by the Program Resources and Logistics Section of the National Cancer Institute. R. Dougherty supplied chicken anti-RAV-1 and anti-RAV-6.

Gamma globulin purification. The separation of hamster IgG was performed according to methods described previously (7, 10). Amounts of 5 ml of hamster anti-gs serum or normal hamster serum were applied to columns (2 by 37 cm) of diethylaminoethyl Sephadex A-50 equilibrated with 0.01 M sodium phosphate buffer, pH 6.5. Gamma globulin was eluted with an NaCl gradient in two peaks. The COFAL activity was recovered quantitatively with 90 to 95% of the CF activity in the first peak.

Radioiodine labeling. Labeling was performed as described previously (10). Iodination efficiencies ranged from 60 to 70% for ^{125}I and 50 to 70% for ^{131}I . The amount of label was approximately 0.5 molecule of I per globulin molecule. Recovery of protein-bound radioactivity was 100%. Residual free iodine was less than 5%. Protein concentrations were determined spectrophotometrically at 280 nm with a bovine serum albumin standard curve.

PRILAT procedures. The methodology for the direct PRILAT has been described previously (10). Hamster anti-gs IgG, labeled with ^{125}I , and normal hamster IgG, labeled with ^{131}I , were mixed in equal protein concentrations and flooded on fixed cover-

slip cultures of test and control cells. Eight or more cover slips of each cell type were used per test, and all tests were repeated three to five times. Cover slips were incubated in a humidified chamber for 1 hr at 37°C , washed in three changes of PBS for 10 min each, air-dried, and counted in a two-channel gamma analyzer. The uptake ratio (UR) of ^{125}I to ^{131}I counts per minute was calculated for each cover slip, and mean values and their deviations were determined. The UR values were used to calculate the specific uptake quotient (SUQ).

The formula for the calculation of the SUQ was

$$\begin{aligned} \text{SUQ} &= \frac{\text{UR on test cells}}{\text{UR on control cells}} \\ &= \frac{{}^{125}\text{I counts per min on test cells}}{{}^{131}\text{I counts per min on test cells}} \bigg/ \frac{{}^{125}\text{I counts per min on control cells}}{{}^{131}\text{I counts per min on control cells}} \end{aligned}$$

The method of Finney (11) was used to calculate the standard errors (SE) of the SUQ values. In the absence of a specific antibody reaction, the expected SUQ value would be 1.00. Values greater than 1.00 would indicate an antibody-antigen reaction. Reactions were accepted as significant when the SUQ minus 2 SE exceeded 1.00; that is, the probability of the SUQ value occurring by chance was less than 0.05.

The methodology for the indirect PRILAT has been described (16). Anti-hamster immunoglobulins (IgG and IgM) were prepared in rabbits by hyperimmunization with hamster globulin purified by agar-block electrophoresis (18). The IgG fraction was labeled with ^{131}I . UR and SUQ values were calculated as for the direct PRILAT.

Inhibition (blocking) of the direct PRILAT procedure has been described (10). In this test, the test and control cells were incubated with the serum under study for 1 hr at 37°C followed by rinsing, and then the direct PRILAT was performed. Inhibition of the direct PRILAT was considered significant when the SUQ value of the uninhibited test exceeded that of the inhibited test by more than 2 SE; that is, the probability of the difference occurring by chance was less than 0.05.

RESULTS

Specificity of the PRILAT for avian gs antigen and antibody. COFAL-positive sera with titers of 1:64 from hamsters (PD-4) bearing SR-RSV-induced tumors were pooled and fractionated, and the IgG was labeled with ^{125}I . Control sera from normal PD-4 hamsters were similarly fractionated and labeled with ^{131}I . The globulins were mixed in equal protein concentrations, and the direct PRILAT was performed on a variety of cells. Significant antibody reactions

occurred with six different cells (Table 1) known to contain avian gs antigen as determined by immunofluorescence tests with gs antibody. This included three chicken and three hamster cell types. The hamster cell lines did not produce RSV and did not contain type-specific virus envelope antigens (12). The SR-RSV-infected chicken cells were virus producers as were the chicken tumor cells. Control cover slips without

cells, but otherwise treated identically, did not bind IgG. In inhibition tests (Table 2), the PRILAT test on gs antigen-positive cells was blocked by COFAL-positive hamster sera but not by normal hamster sera. The COFAL-positive hamster sera did not contain neutralizing antibodies to SR-RSV.

Comparative gs antigen content of cells. The direct PRILAT was used to estimate the relative

TABLE 1. Comparison of the avian leukosis group-specific (gs) antibody absorptive capacity of avian gs antigen-containing hamster and chicken cells as determined by direct PRILAT^a

Cell type ^b	Mean ¹²⁵ I counts/min	Mean ¹³¹ I counts/min	Uptake ratio	SUQ ^c ± 2 SE	P	Molecules ^d of ¹²⁵ I-IgG/cell	Molecules ^d of ¹³¹ I-IgG/cell	Ratio of ¹²⁵ I- to ¹³¹ I-IgG/cell ^e
Hamster cells								
BHK-21.....	5,341	1,083	4.93	—		7.8 × 10 ⁶	7.2 × 10 ⁶	1.08
H-RSV(BH).....	6,981	1,039	6.72	1.36 ± 0.19	<0.01	10.3 × 10 ⁶	6.9 × 10 ⁶	1.49
SRHT.....	7,743	1,127	6.87	1.39 ± 0.22	<0.01	15.2 × 10 ⁶	10.0 × 10 ⁶	1.52
BHK-RSV(SR).....	8,971	1,256	7.14	1.44 ± 0.13	<0.01	13.2 × 10 ⁶	8.3 × 10 ⁶	1.59
Chicken cells								
CEF.....	6,000	1,249	4.80	—		14.1 × 10 ⁶	13.2 × 10 ⁶	1.07
CT.....	10,328	1,293	7.98	1.66 ± 0.25	<0.01	30.3 × 10 ⁶	17.2 × 10 ⁶	1.76
CEF-RSV-RAV-50...	9,603	1,163	8.25	1.72 ± 0.18	<0.01	37.6 × 10 ⁶	19.6 × 10 ⁶	1.83
CEF-SR-RSV.....	11,017	1,233	8.93	1.86 ± 0.20	<0.01	43.2 × 10 ⁶	21.9 × 10 ⁶	1.97

^a The antibody (PRILAT) mixture contained 100 µg of anti-gs-antigen ¹²⁵I-labeled IgG/ml and 100 µg of normal ¹³¹I-labeled IgG/ml. Specific activities were 10⁶ counts per min per µg of ¹²⁵I and 6.3 × 10⁴ counts per min per µg of ¹³¹I. Calculations of IgG absorbed per cell were adjusted for background and radiodecay after iodination.

^b Primary chicken embryo cells (CEF) were infected with 1 plaque-forming unit/cell of RSV and fixed 48 hr postinfection. H-RSV(BH) = Bryan-RSV Hamster Tumor Cells grown in vitro. SRHT = Schmidt-Ruppin RSV hamster tumor cells grown in vitro. BHK-RSV(SR) = Schmidt-Ruppin-RSV-transformed BHK-21 cells. CT = Schmidt-Ruppin RSV chicken tumor cells.

^c Specific uptake quotient, calculated with BHK-21 data as the denominator for the hamster cells, and CEF as the denominator for the chicken cells.

^d Molecules of IgG absorbed per cell =

$$\frac{(\text{mean counts per min per cover slip})(3.76 \times 10^{12} \text{ molecules per } \mu\text{g of IgG})}{(\text{mean cells per cover slip})(\text{specific activity in counts per min per } \mu\text{g of IgG})}$$

^e Micrograms of ¹²⁵I-IgG/micrograms of ¹³¹I-IgG per cell.

TABLE 2. Specificity of the PRILAT for avian leukosis virus group-specific antigen as determined by direct PRILAT inhibition (blocking) experiments

No. of consecutive incubations with unlabeled serum prior to direct PRILAT ^a	Normal hamster serum ^b		Immune hamster serum ^c	
	SUQ ± 2 SE	P	SUQ ± 2 SE	P
0	1.61 ± 0.25	<0.01	1.61 ± 0.25	<0.01
1	1.42 ± 0.11	<0.01	1.11 ± 0.09	<0.05
2	1.34 ± 0.08	<0.01	1.03 ± 0.14	
3	1.29 ± 0.12	<0.01	0.98 ± 0.12	
4	1.26 ± 0.14	<0.01	0.96 ± 0.21	

^a SR-RSV-induced chicken tumor cells and chicken embryo fibroblasts grown on cover slips were incubated for 0.5 hr with sera diluted 1:10 in PBS and washed; then they were reincubated with fresh serum and the PRILAT was performed.

^b Pool of 10 sera from normal control hamsters.

^c Hamster antiserum with a COFAL titer of 1:8.

gs antigen content of cells based on the amount of antibody taken up per cell. These calculations were made on the cells tested in Table 1. Final values, molecules of IgG absorbed per cell, were calculated by use of the formula given in Table 1. The counts per minute data were corrected for background and radioisotope decay after iodination.

Under ideal conditions of a perfectly matched PRILAT mixture, the ratio of ^{125}I -labeled IgG and ^{131}I -labeled IgG on control cells should equal 1.00. Actual values obtained on the gs antigen-negative cells were 1.08 on BHK-21 cells and 1.07 on primary CEF cells. These deviations from 1.00 were not significant.

In Table 1, the cells of each animal species are listed from top to bottom according to the increase in SUQ value. Since antibody binding is generally proportional to antigen content, it follows that the sequence in Table 1 should be a fair estimate of the comparative gs antigen content of the cells studied. A test of this estimate is provided by comparing the SUQ values with the calculated IgG binding ratios for each cell type. Since SUQ values correct for non-specific binding of normal and immune globulin on test and control cells, it should be expected that the higher the SUQ value the higher the proportion of immune IgG bound. As can be seen in Table 1, the SUQ values reflected this relationship; thus, the comparative gs antigen content of the various cells as predicted by SUQ values and by the relative amount of gs antibody bound per cell are in agreement. Since the virus-producing chicken cells bound more antibody per cell than the virus-free hamster cells, it would appear that they contain greater amounts of gs antigen. This was confirmed in immunofluorescence and CF tests.

Detection of gs antibody in COFAL-negative sera from hamsters bearing SR-RSV-induced tumors. From preliminary COFAL tests on 150 sera from RSV-tumor-bearing hamsters, 9 negative sera were obtained. These sera together with known positive and negative control hamster sera were tested undiluted in direct PRILAT inhibition tests on chicken tumor cells (Table 3). In these tests, the SUQ value of the direct PRILAT for gs antibody was 1.58. Normal hamster sera in the inhibition test reduced this value by 10 to 13%, and the nine COFAL-negative sera from tumor-bearing hamsters reduced the SUQ by 23 to 58%. Per cent reduction of SUQ was calculated as follows: expected SUQ (1.58) minus observed SUQ divided by expected SUQ $\times 100$. These results were considered as presumptive evidence of gs antibody in all nine sera, even though the conclusion with sera 111 and 115 might be ques-

TABLE 3. *Detection by direct PRILAT inhibition of avian leukosis group-specific antibody in COFAL-negative sera from SR-RSV-induced-tumor-bearing hamsters*

Blocking sera ^a	SUQ $\pm$ 2 SE	P	Per cent reduction ^b of SUQ
None ^c	1.58 $\pm$ 0.08	<0.001	—
Normal hamster ^d	1.40 $\pm$ 0.13	<0.01	10–13
13	0.78 $\pm$ 0.14	0.05	51
70	1.16 $\pm$ 0.15		27
81	1.08 $\pm$ 0.10		32
101	0.82 $\pm$ 0.11		48
111	1.21 $\pm$ 0.28		23
115	1.02 $\pm$ 0.31		35
117	0.86 $\pm$ 0.15		58
123	0.83 $\pm$ 0.19		47
125	1.03 $\pm$ 0.11		35

^a Chicken tumor and chick embryo fibroblast cover slips were incubated with undiluted sera for 0.5 hr prior to direct PRILAT tests.

^b Per cent reduction was calculated from the formula: (unblocked SUQ — blocked SUQ) $\times$ (100) $\div$ unblocked SUQ.

^c Regular direct PRILAT performed with hamster IgG with a COFAL titer of 1:8.

^d Four normal hamster sera were tested to establish the range of variation.

tioned because of the high SE associated with the mean SUQ values.

To determine the nature of blocking activity in the COFAL-negative sera, the IgG fraction was prepared from a 3-ml pool of the sera from hamsters 70, 101, and 123. Serial dilutions of IgG were tested for gs antibody by indirect PRILAT and by blocking of the direct PRILAT (Table 4). Identical tests were performed with normal hamster IgG prepared similarly.

In these direct PRILAT blocking tests, the antibody reaction was considered highly significant ($P < 0.01$) when the normal SUQ minus 2 SE still exceeded the immune plus 2 SE. In indirect PRILAT, the antibody reaction was considered highly significant ($P < 0.01$) when the immune SUQ minus 2 SE exceeded the normal SUQ plus 2 SE. Based on these criteria, the titer of the immune IgG was 1:8 < 1:32 in both tests.

In further studies, the gs antibody titer of COFAL-negative hamster sera was determined by direct PRILAT inhibition tests. Figure 1 presents the results of titration of sera 70, 101, and 123 as listed in Table 3. The gs antibody titers of these sera were 1:2, 1:128, and 1:8, respectively.

These studies, which employed several forms of the PRILAT, clearly identified avian leukosis

TABLE 4. Titration of hamster IgG from COFAL-negative sera for antibody to avian leukosis group-specific antigen by direct PRILAT inhibition and by indirect PRILAT^a

Test	Source of hamster IgG ^b	SUQ $\pm$ 2 SE with IgG diluted				Titer
		1:1	1:8	1:32	1:128	
Direct PRILAT Inhibition	Normal	1.20 $\pm$ 0.08	1.23 $\pm$ 0.06	1.19 $\pm$ 0.13	1.24 $\pm$ 0.14	1:8 < 1:32
	Tumor-bearing	0.98 $\pm$ 0.08 ^c	0.95 $\pm$ 0.08 ^c	1.02 $\pm$ 0.11	1.12 $\pm$ 0.10	
Indirect PRILAT	Normal	0.92 $\pm$ 0.07	0.76 $\pm$ 0.10	1.12 $\pm$ 0.14	1.01 $\pm$ 0.12	1:8 < 1:32
	Tumor-bearing	1.23 $\pm$ 0.16 ^c	1.16 $\pm$ 0.15 ^c	1.12 $\pm$ 0.14	1.20 $\pm$ 0.06	

^a Components for the direct PRILAT were described in Table 1. The secondary reagent for the indirect PRILAT contained 200 μ g/ml of ¹²⁵I-labeled rabbit IgG anti-hamster IgG and 205 μ g/ml of ¹³¹I-labeled normal rabbit IgG. Specific activities were 3.9×10^4 counts per min per μ g of ¹²⁵I-IgG and 7.8×10^4 counts per min per μ g of ¹³¹I-IgG.

^b Normal hamster IgG from PD-4 hamsters not injected with SR-RSV. IgG from a pool of sera from tumor-bearing hamsters 70, 101, and 123 listed in Table 3.

^c The differences between these SUQ values and those of the corresponding control (normal) were highly significant ($P < 0.01$). Differences in SUQ values at 1:32 and 1:128 dilutions were not significant at the $P = 0.05$ level.

FIG. 1. Inhibition of the direct PRILAT for avian group-specific antigen by COFAL-negative sera from hamsters bearing SR-RSV tumors. Titers of individual sera were as follows: 70, 1:2; 101, 1:8; and 123, 1:128. See text for methodology.

gs antibody in COFAL-negative sera from hamsters bearing SR-RSV-induced tumors.

Examination of avian sera for avian leukosis gs antibodies by PRILAT. In view of the preceding demonstration of gs antibodies in COFAL-negative hamster sera and the reports by Rabbotti and Blackham (19), by Armstrong (2), and by Roth et al. (20) that certain avian sera contain gs antibody, PRILAT tests for gs antibody were conducted on sera from chickens with SR-RSV-induced tumors and on sera from fowl immunized with various avian viruses (Table 5). The latter sera were known to contain specific

avian leukosis neutralizing antibodies and were supplied by P. Sarma, by R. Dougherty, or through the auspices of the Program Resources and Logistics Section of the National Cancer Institute.

Direct PRILAT inhibition and indirect PRILAT were employed. In each test, evidence of antibody activity was accepted when the difference in SUQ values between normal sera and test sera was greater than 2 SE. A summary of results is shown in Table 5.

The reference hamster antisera, known to contain gs antibodies, were strongly positive by direct PRILAT inhibition and by indirect PRILAT. The pigeon antiserum, tested only by direct PRILAT inhibition, was also strongly reactive at a 1:100 dilution. The chicken and turkey antisera reacted in one or both PRILAT tests, but titers, usually 1:10, were markedly lower than with the hamster and pigeon antisera. No gs antibodies were detected in sera obtained from 10 SPAFAS chickens bearing wing-web tumors induced with SR-RSV. These results indicate that production of avian leukosis gs antibodies can be induced in chickens and turkeys. These studies are continuing.

DISCUSSION

These studies were predicated on the basis that the PRILAT would provide a highly sensitive means to detect antigen-antibody reactions (10, 16, 17). Thus, it was not unexpected that the PRILAT would reveal antibodies to avian leukosis gs antigen(s) in sera from tumor-bearing hamsters which were negative by direct CF (COFAL). Previous studies (10, 16) had demon-

TABLE 5. *PRILAT* tests for avian leukosis group-specific (gs) antibodies in reference to hamster and avian antisera

Antiserum	Source ^a	Log -10 neutralization index	Avian leukosis gs antibody titers by		
			COFAL test	Direct PRILAT inhibition ^b	Indirect, PRILAT ^b
Hamster anti-gs (SR-RSV-D)	Tumor-bearing PD-4 hamsters	<0.4	≥64	>256	>100
Hamster anti-gs	P. Sarma	NT ^c	64	>256	>100
Pigeon anti-gs (SR-RSV-B)	P. Sarma	>2.0	80	>100	NT
Chicken anti-RAV-1	R. Dougherty	>2.0	NT	10 < 100	NT
Chicken anti-RAV-2	PRLS-NCI	>2.0	NT	10 < 100	10
Chicken anti-CZ-RSV	PRLS-NCI	>3.0	NT	10 < 100	NT
Normal chicken	SPAFAS	<0.4	NT	<10	<10
Chicken anti-RAV-6	R. Dougherty	3.7	NT	10 < 100	NT
Turkey anti-B-RSV	PRLS-NCI	>4.0	NT	10	<10
Turkey anti-SR-RSV	PRLS-NCI	2.0	NT	10	10
Turkey anti-H-RSV	PRLS-NCI	>2.0	NT	10	10
Turkey anti-RAV-1	PRLS-NCI	>4.0	NT	10	<10
Turkey anti-RSV-559	PRLS-NCI	>2.0	NT	10	10
Normal turkey	PRLS-NCI	<0.4	NT	<10	<10
Ten SPAFAS chickens given SR-RSV	All tumor-bearing	NT	NT	<10	<10

^a PRLS-NCI = Program Resources Logistics Section, National Cancer Institute.^b These tests were conducted with BHK-RSV(SR) as gs positive cells and BHK-21 as control cells.^c Not tested.

strated adenovirus 12 T antibody in CF-negative sera from hamsters bearing adenovirus 12 tumors (10).

It was also anticipated that gs antibodies might be detected in sera of chickens and turkeys immunized with avian leukosis viruses. This possibility seemed likely on the basis of reports that sera from immunized chickens, although COFAL-negative, reacted with gs antigen(s) in immunodiffusion (2) and by CF inhibition (19, 20). These latter two tests as well as the PRILAT are not complement-dependent tests and hence will detect antigen-antibody reactions that do not fix guinea pig complement. Since it is not known whether the gs antibodies in chicken and turkey sera will fix homologous complement, one cannot yet attribute their demonstration by PRILAT or by CF inhibition solely to the greater sensitivity of these two tests as compared with direct CF. Armstrong (2) found precipitating antibody to one component of the gs complex (2, 9, 12) in only 8 of 72 chicken antisera but predicted that more sera might be positive with more sensitive serological tests. Rabotti and Blackham (19) detected gs antibodies by CF inhibition in apparently all of 34 chicken and turkey antisera with type-specific virus neutralizing antibody titers of 1:128 or greater. Their gs antibody titers were 1:8 to 1:32, but a few titered 1:64. Roth et al. (20)

recently confirmed that chickens immunized with various leukosis viruses produce gs antibodies as detected by CF inhibition. In the present study, four sera from immunized chickens and five from immunized turkeys contained gs antibodies as determined by PRILAT. Titers were low, usually 1:10. These studies are being continued with antisera prepared in a larger number of chickens. At this point, it appears that our preliminary results confirm that gs antibodies can be produced by chickens and turkeys.

In addition to the several gs virion components which may participate to varying degrees in the PRILAT, there may also be nonstructural gs antigens which occur only in infected cells and may bind corresponding components of IgG. Studies on tumor-specific transplantation antigens indicate the existence of such non-virion gs antigens in cells transformed by avian tumor viruses (3). The PRILAT as employed herein did not discriminate between virion and nonvirion gs antigens.

These studies confirmed the value of the direct PRILAT as a means to measure the relative antigenic content of cells based on the amount of antibody absorbed per cell. Non-virus-producing (12, 23) RSV-transformed hamster cells or tumor cells appeared to contain less gs antigen per cell (on a mean basis) than did SR-RSV-infected chicken embryo fibroblasts or

SR-RSV-induced chicken tumor cells. Nonetheless, the hamster cells contained sufficient amounts of gs antigen and were used in the tests for gs antibody in avian antisera. The use of these hamster cells, which did not contain viral envelope antigens (12, 23), eliminated the possibility that the antibodies detected in the avian antisera were viral envelope antigens.

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