acclimation and de-acclimation on the immune response in 8-week-old mice was conducted. The mice were exposed to an ambient temperature of 4°C for 4 weeks and some of these were then de-acclimated at room temperature (23°C) for an additional 4 weeks. In each case, controls were maintained at 23° C. The antigen used was coliphage T_2r^+ and the levels of circulating antibody were determined in terms of the remaining serum plaque-forming units of coliphage. Neither acclimation nor de-acclimation, under these conditions, resulted in significant change in the levels of circulating coliphage antibodies.

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Modification of Complement Fixation Test for Estimation of Rous Sarcoma Virus Antibodies in Turkey and Chicken Serums. (27797)

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Bushnell and Hudson(1) and Rice(2) have reported on attempts to use the classical complement fixation (C-F) test for chicken serum but found that some heated serums were anticomplementary at dilutions of 1:50 and lower. More recently Brumfield and Pomeroy(3), in tests with ornithosis antigen and unheated turkey serums, reported adequate qualitative results by increasing the amount of complement to overcome the anticomplementary effects; however, many serums could be used only at relatively high dilutions. Later they(4) found that enhanced fixation of guinea pig complement occurred when heat inactivated serums were supplemented with small amounts of fresh, normal chicken or turkey serums. Boulanger and Bannister(5) reported good fixation of guinea pig complement with heated bovine serum and vesicular stomatitis virus when fresh, normal calf serum was added. Recently Benson, *et al.*(6) and Brumfield, *et al.*(7) showed that the factor in normal, unheated chicken serum was probably the C'1 component of chicken complement.

This is a brief report on the detection and quantitation of Rous sarcoma virus antibody in heated turkey and chicken serum with a modified C-F test.

Materials and methods. Antigen. Rous sarcoma virus (RSV) antigen used in these studies was prepared according to the procedures described by Moloney(8), except that protamine sulfate precipitation was not done and the 40,000 × *g* pellet was brought back into suspension with 0.05 M sodium citrate, pH 6.7, to a concentration of one gram of original tumor per milliliter of final material.

Antiserums. The anti-Rous virus chicken serums were produced by the method of Fink and Rauscher(9). The anti-Rous virus turkey serums were produced by subcutaneous inoculation of 7-day-old turkey poults with

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0.2 ml of a 10^{-4} dilution of the above RSV preparation. Tumors appeared in approximately 3 weeks, progressed relatively slowly until they attained a size of approximately 50 mm in diameter in about 3 months, after which they regressed. The turkeys were exsanguinated at 4 months post-inoculation and the sera were collected and stored at –20°C.

Sheep erythrocytes. Whole blood was collected aseptically in equal volumes of a modified Alsever's solution(7), mixed thoroughly and stored at 4°C. Immediately before use, the cells were washed 3 times in veronal buffer and brought back to a 2% suspension.

Normal chicken serum and C'1 component of normal chicken serum complement. Normal chicken serum (Nch) and the C'1 component of normal chicken serum complement were obtained from 3 months old White Leghorns of line 15I, an isolated stock whose sera were tested for Rous sarcoma virus antibody (RSVA) by neutralization in tissue culture (T-C) as outlined by Rubin(10) and found free of RSVA activity. The sera were collected, pooled, clarified by centrifugation and stored at –70°C until used. In the C-F test with turkey serum Nch was used at a level of 5% (v/v) of the guinea pig complement aliquot. The Nch C'1 used in testing of chicken serums was obtained by the procedure described by Brumfield, *et al.*(7). This concentrate was incorporated into the test system at a level of 5% (v/v) of the guinea pig complement aliquot.

Hemolysin and complement. Commercial hemolysin and guinea pig complement (Difco) were used throughout these studies.

The C-F test. The reagents were added in the following sequence to give a total of 1.0 ml in each 10 × 70 mm test tube:

1) 0.2 ml of serum; 2) 0.2 ml of guinea pig complement containing 2 exact units and the Nch factor or factors; 3) 0.2 ml of antigen diluted to contain 2 units (100,000 focal-forming-units (FFU).

This mixture was well shaken and allowed to react for 4 hours at 4°C, following which 0.4 ml of a 2% sheep erythrocyte suspension sensitized with an equal volume of hemolysin (2 units per test portion) was added. The final incubation was carried out at 37°C in a water bath, and the reactions were read in 30 minutes, or sooner if both serum and antigen controls were completely hemolyzed.

TABLE I. Influence of Incubation Time on Complement Fixing Activity of an Anti-Rous Sarcoma Virus Turkey Serum.

Incubation time (hr)	Incubation temp (°C)	C-F titer
1	37	<1: 10
1	4	<1: 10
2	4	1: 40
4	4	1:640
8	4	1:640
12	4	1:640

A negative control serum gave no fixation of complement at any of the above incubation times.

Results. The results of a preliminary study on the influence of incubation time on C-F activity of an anti-Rous sarcoma virus turkey serum are given in Table I. Only traces of fixation were observed after incubation at 37° or 4°C for 1 hour, and low titers were obtained at 4°C after 2 hours. However, a maximum titer was obtained after incubation for 4 hours at 4°C, and no further increase was observed by extending the incubation to 8 or 12 hours.

The neutralization and complement fixation titers of 16 turkey sera for RSV are given in Table II. The neutralization activity is given in terms of the greatest dilution of serum which neutralized 90% or more FFU of RSV. The C-F titers recorded were the greatest dilution of serum which gave a 2+ or greater fixation of complement. When these terms are used the apparent sensitivity of the C-F test is less than the neutralization test;

TABLE II. Comparative Rous Sarcoma Virus Tissue Culture Neutralization and Complement Fixation Activity Titers of Turkey Sera.

Serum No.	C-F titer	Neutralization titer	Serum No.	C-F titer	Neutralization titer
457	1:640	1:100,000	4312	1:40	1:1,000
4313	1:160	1: 10,000	1850	1:40	1:1,000
4309	1:160	1: 10,000	4306	1:40	1:1,000
4298	1:160	1: 1,000	4295	1:40	1: 100
4300	1:160	1: 1,000	4321	Neg.	Neg.
4297	1: 40	1: 10,000	4318	Neg.	Neg.
1853	1: 40	1: 1,000	1837	Neg.	Neg.
1841	1: 40	1: 1,000	1843	Neg.	Neg.

A pool of the above positive serums was tested against normal chick embryo antigen and was negative in the C-F test.

TABLE III. Influence of Varying Concentrations of Normal Heat Inactivated Chicken Serum Used as a Supplement in Complement Fixation Test.

Serum No.	% concentration of NchH*	Serum dilution 1:10	1:20	1:40	1:80	1:160	1:10†
1495 (+)	0	4‡	4	4	4	4	0
	2	4	3	2	0	0	0
	4	4	3	2	2	0	0
	5	4	3	1	0	0	0
1435 (+)	0	4	4	4	4	4	0
	2	4	3	1	0	0	0
	4	4	4	2	2	0	0
	5	4	4	2	0	0	0
12159 (−)	0	0	2	3	4	4	0
	2	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0

* Percent concentration of normal heat-inactivated chicken serum in addition to that which was added as the suspending fluid for C'1 concentrate (5%).
† Serum control, no antigen added.
‡ Degree of fixation: 4 = complete fixation, 0 = no fixation.

however a good positive correlation was obtained. The last 4 sera in the table were from uninoculated control turkeys. The only primary discrepancy was with serum No. 4297 which gave a titer of 1:10,000 with the neutralization test and only 1:40 with the C-F test. However, this discrepancy may not be as large as it appears, since the serial serum dilutions were 10-fold in the former and 4-fold in the latter. A shift of only one dilution in either test result would have improved the correlation of titers by the two methods. A pool of the positive sera was negative when tested against normal chick embryo antigen.

In a study to determine the amount of chicken C'1 concentrate and heated Nch to use in the chicken C-F test it was found that a 3 times concentrated C'1 used at a concentration of 5% in relation to the guinea pig complement aliquot was optimum. Lower concentrations were inadequate to fix complement whereas higher concentrations were anticomplementary. In addition it was necessary to increase the quantity of heated Nch by 2 to 5% in relation to the guinea pig complement aliquot, since lesser amounts were inadequate to overcome the nonspecific reaction. Specific fixation was detectable only when heated Nch was present in adequate concentrations. This is clearly shown with serum No. 12159, a negative control serum (Table III), which was negative at a dilution of 1:10 but gave a nonspecific fixation at higher dilutions when no additional heated Nch was added. However, when 2% or greater amounts of heated Nch were added, the nonspecific fixation was eliminated. Sera No. 1495 and 1435, on the other hand, showed fixation at 1:10 and greater dilutions when no additional heated Nch was added, but specific fixation was clearly defined when 2% or greater amounts of heated Nch were added.

The anti-Rous virus titers of 14 chicken sera by the C-F and neutralization tests are given in Table IV. Here again, as in the titrations with RSV antigen and turkey antiserum, fairly good correlation was obtained. However, 3 of the sera showed a negative C-F test although the neutralization titers were quite high, and conversely, serum No. 1421 showed C-F activity but no neutralizing activity.

Discussion. It is well known that by ex-

TABLE IV. Comparative Antibody Titers of Some Anti-Rous Virus Chicken Sera by C-F and Neutralization Tests.

Serum No.	C-F titer	Neutralization titer	Serum No.	C-F titer	Neutralization titer
1748	1:320	1:100	1730	Neg.	1:160
1425	1:160	1:100	1419	Neg.	1:100
1495	1: 80	1:100	1742	Neg.	1: 10
1424	1: 80	1:100	1494	Neg.	Neg.
1738	1: 40	1:640	1746	Neg.	Neg.
1575	1: 10	1:160	1750	Neg.	Neg.
1421	1: 10	Neg.	1740	Neg.	Neg.

tending the incubation period of initial C-F antigen-antibody mixtures in the cold, a greater fixation is obtained. As a result, however, the specificity of the reactions is greatly decreased. Studies are contemplated to determine the specificity of the test employing various incubation temperatures and incubation times.

Brumfield, *et al.*(7) and Jeon(11) have shown that additional quantities of chicken C'1 are required for initiation of the complement fixation reaction when chicken serum is employed. This requirement for additional chicken C'1 has been confirmed for the Rous sarcoma virus-antibody system in trials with 14 chicken serums. In addition, however, an increased requirement for heated Nch was also noted (Table III). The component in heated Nch appears to be a lytic factor, possibly C'3 or one of its components, since it is present in the inactivated negative test serum (serum No. 12159, Table III).

The importance of the order of addition of reagents for obtaining maximum titers has been emphasized by Brumfield, *et al.*(7). With the RSV antigen-turkey and chicken antiserum systems, the sequence of reagent addition did not greatly influence the titers obtained. In fact, slightly higher titers were noted using the standard sequence when Nch or C'1 concentrate was added to guinea pig complements as described under *Materials and methods*.

Summary. A complement fixation test with Rous sarcoma virus is described and some preliminary results reported. Although the sensitivity of the test appears to be somewhat lower than *in vitro* tissue culture neutralization tests, the correlations obtained are quite good.

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Effect of Deuterium Oxide Incorporation of P^{32} into Rat Liver Deoxyribonucleic Acid.* (27798)

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In recent years considerable attention has been given to the physiological and biochemical effects of deuterium in mammals. Besides gross physiological effects such as hyperexcitability, loss of weight, ulceration of the skin, and eventual death, effects on the functions of vital organs and on metabolic processes have been observed(1-3). Experiments both *in vivo* and *in vitro* have shown that substitution of deuterium for hydrogen in various enzyme systems generally results in a reduction of reaction rates. Although this is not always true, it is commonly expected in reactions

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