Prevalence of Reticuloendotheliosis in Chickens: Immunofluorescence Studies (33516)

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The virus of reticuloendotheliosis (REV)[3] was isolated in 1958 from an outbreak of a disease in turkeys (1). Although its pathogenicity for certain fowl was investigated (2, 3), little is known of its natural prevalence. This report describes our experience with REV as an antigen in the indirect immunofluorescence test which we used to check on the occurrence of specific antibody in chicken eggs from different areas of the United States.

Materials and Methods. Viral antigens. Reticuloendotheliosis virus was kindly provided by Dr. K. M. Cook, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, NIH. It was maintained in our laboratory by serial passage in cultured chick embryo fibroblasts (CEF), derived from RIF-free and mycoplasma-free eggs (NIH flock).

Three strains of Rous sarcoma virus were used: Schmidt-Ruppin (subgroup B) lot TV43, Harris (subgroup B) lot HA8, and RSV(RAV-1) (subgroup A) lot TC-A-1; all were kindly supplied by Dr. R. Holdenried, National Cancer Institute, NIH. One of the avian leukosis viruses RIF,[4] strain RPL-12-L37, was supplied by Dr. W. Okasaki, Regional Poultry Laboratory, East Lansing, Michigan. The Rous sarcoma viruses and the avian leukosis virus will be hereafter referred to as sarcoma–leukosis virus (SLV) group.

Newcastle disease virus (NDV), strain Kemerovo No. 98, was obtained from the Arbovirus Unit of this laboratory. The CEF coverslip preparations for each virus antigen were prepared essentially as previously described (4).

Specific immune sera. Antisera were prepared for REV, RSV(RAV-1), and NDV by immunization of 60 week-old RIF-free roosters, whose preimmunization sera were without demonstrable antibodies to these antigens when tested at 1:40 dilution. The sera against Schmidt-Ruppin and Harris strains of SLV groups were available through the courtesy of Dr. R. Holdenried.

Survey eggs. Eggs were obtained in the spring of 1967 through the courtesy of Mr. B. W. Kempers, Consumer Marketing Service, Poultry Division, U.S. Department of Agriculture, from 92 chicken flocks in 12 states (see Fig. 1). The eggs were from hens 1 or more years of age; all the eggs from a given flock were collected on the same day. Immediately upon receipt at NIH the eggs were refrigerated at 4°. Yolk antibody extracts were prepared within 1–2 weeks and stored in Revco freezers at −60° until tested by indirect immunofluorescence (4).

Indirect immunofluorescence technique. The antibody extract, designated a 1:2 dilution of the yolk, was diluted fourfold (making a 1:8 yolk dilution) and added to the coverslip preparation. Details of the staining procedure and examination for antibody were previously described (4).

Results and Discussion. Utilizing indirect immunofluorescence methodology, we compared the reactions produced by REV with other common chicken viruses and their respective specific immune sera. The REV immunofluorescence pattern while immunologically specific was by no means unique in its appearance. In fact, on occasion, one would be hard put to differentiate the intensely staining cytoplasmic granules of REV-

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[3] Also known as T-virus or Twiehaus virus.

[4] RIF = resistance-inducing factor.

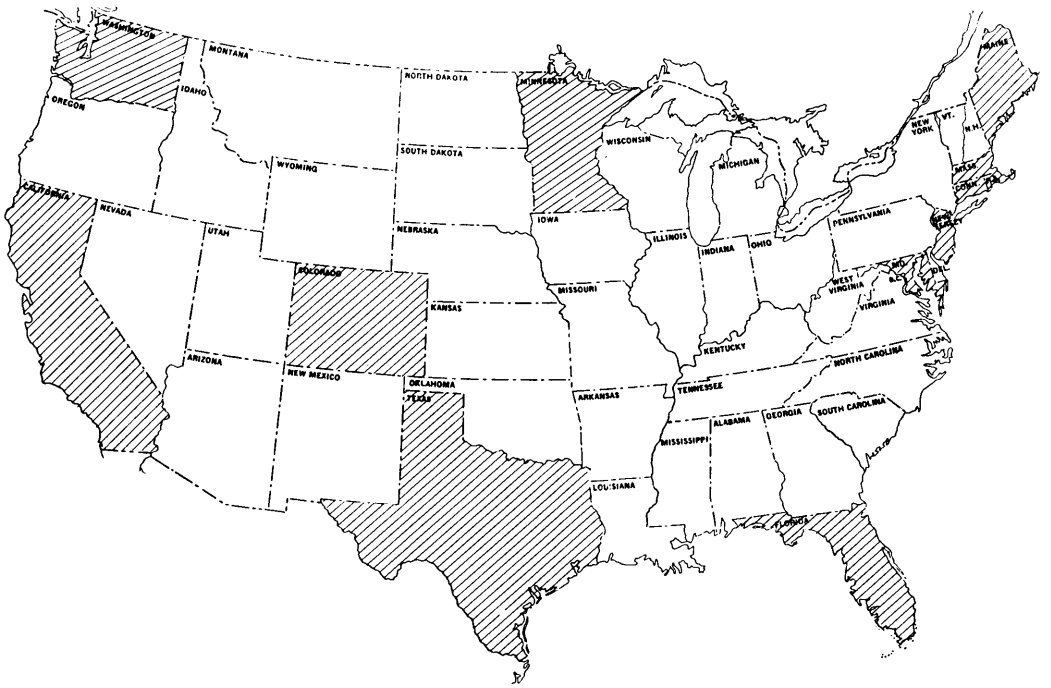

FIG. 1. Chicken egg collection sites: 92 flocks in 12 states.

infected cells from those produced by SLV, such as RSV(RAV-1), or from certain non-oncogenic avian viruses, such as NDV.

Table I summarizes the results of a cross-immunofluorescence test performed to check on the specificity of the REV reaction. The 4 members of the SLV group cross-reacted although to a variable extent, but none of them reacted with REV. Neither REV nor SLV reacted with NDV control reagents. Thus, it appeared that REV was antigenically distinct from the SLV group, as already reported by others (1,2,3,5). Furthermore, apparently indirect immunofluorescence could

TABLE I. Results of Cross-Immunofluorescence Tests with Reticuloendotheliosis Virus and Sarcoma–Leukosis Viruses.

	Antiserum[a]				
		SLV			
Antigen	REV	RSV(RAV-1)	RSV-H	RSV-SR	NDV
REV[b]	4+[e]	0	0	0	0
SLV[c] RSV(RAV-1)	0	4+	1+	1+	0
RSV-H	0	1+	4+	4+	0
RSV-SR	0	1+	4+	4+	0
RIF	0	2+	4+	4+	0
NDV[d]	0	0	0	0	4+

[a] A 1:40 dilution.

[b] Reticuloendotheliosis virus.

[c] Sarcoma–leukosis viruses.

[d] Newcastle disease virus.

[e] Numerical rating based upon degree of brilliance—4+ indicates maximal brightness and zero indicates absence of specific fluorescence.

be used to detect the presence of the specific REV antibody.

An opportunity to put this procedure to test presented itself in the course of our studies on the usefulness of a recently developed immunofluorescence test (4) for the detection of microbial antibody in chicken eggs (6). Yolks from 905 eggs were extracted for antibody and tested at 1:8 dilution with the REV antigen grown in CEF. The eggs were collected from 92 flocks in 12 states (Fig. 1). The results are summarized in Table II.

TABLE II. Results of Immunofluorescence Survey of Chicken Eggs from 12 States for Antibody against RE Virus Antigen.

State	Flocks with ab[a] /flocks tested	No. of eggs with ab[a] /no. of eggs tested
California	0/13	0/67
Colorado	2/12	4/125
Connecticut	0/3	0/34
Florida	7/12	24/106
Maine	0/2	0/33
Maryland	2/2	9/24
Massachusetts	2/4	7/41
Minnesota	7/12	25/136
New Jersey	4/10	11/100
Rhode Island	1/1	3/12
Texas	12/12	55/129
Washington	4/9	9/98
Total positive/ total tested	41/92	147/905

[a] ab = antibody for RE virus antigen.

Almost half the flocks tested were infected with REV and about 16% of the individual eggs contained the antibody. As would be expected with the small samples, the proportion of antibody-containing eggs varied from state to state and from one area to another within a state. Nevertheless, it is clear that this infection must be common in chicken flocks throughout the United States.

Chickens of different genetic stocks appeared to be equally susceptible to REV infection: 125 of 713 white eggs and 22 of 192 brown eggs contained the antibody. About 50% of the eggs with REV antibody also contained antibody to the SLV antigens.

The existing lack of information on the natural prevalence of RE viral infection in chickens and other domestic fowl possibly has been due to the relatively recent announcement of the initial isolation of this virus. Sevoian *et al.* reported in 1964 that the virus was isolated by Twiehaus in 1958 from a flock of turkeys with leukosis-like lesions. They also reported that the infection of chicken embryos and 1- to 3-day-old chicks of several genetic lines with the Twiehaus virus produced after a brief incubation period a fatal disease characterized by enlargement of the liver and spleen with proliferation of primitive mesenchymal cells, especially around the tunica adventitia and the small arterioles. Sevoian *et al.* classified the virus as a member of the avian leukosis complex and named it the T-virus.

Subsequently the same workers (7) showed that the characteristic lesions in the chicken embryo could be prevented by mixing the T-virus with serum or egg yolk obtained from immunized hens. They also reported propagation of the virus in cultured CEF and showed that the virus did not possess RIF activity.

Theilen *et al.* (2) produced a disease in newly hatched chickens, turkeys, and Japanese quail, characterized by discoloration and enlargement of the liver with proliferation of reticuloendothelial cells in the hepatic sinusoids and around blood vessels. Unlike Sevoian *et al.* they considered these lesions to be consistent with reticuloendotheliosis (RE) rather than lymphomatosis. Zeigel *et al.* (3) demonstrated by electron microscopy that this virus is morphologically different from the viruses of avian leukosis group. On the basis of different histopathology, morphology, and the lack of both RIF and COFAL[4] activity, the virus was designated *RE virus (strain T)*, distinct from the sarcoma–avian leukosis group of viruses.

Summary. Prevalence of natural infection of chickens with reticuloendotheliosis virus (REV) was explored using egg yolk extract as the source of antibody in the indirect immunofluorescence reaction. One-hundred

[4] COFAL = complement-fixation test for avian leukosis.

forty-seven of 905 eggs from 41 of 92 chicken flocks in 12 states contained the antibody. Although there was considerable geographic variation consistent with sampling problems, it is apparent that this is a common virus infection of North American chickens. Results of the cross-immunofluorescence test confirmed that REV is not a member of the well-characterized sarcoma-leukosis group of avian viruses.

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Effect of Statolon on Acute and Persistent Murine Leukemia and Sarcoma Virus Infections (33517)

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Statolon, a product of a mould, *Penicillium stoloniferum*, has been shown to exert its antiviral action by stimulating the *in vitro* and *in vivo* production of interferon (1,2). Statolon, now known as double-stranded phage RNA (3, 4), was recently reported to inhibit Friend leukemia virus infection in mice (5, 6). Statolon inhibits Friend leukemia virus when administered as early as 6 days before, and as late as 9 days after, virus inoculation. The majority of statolon-treated mice are completely protected against Friend virus leukemia and are resistant to rechallenge with the virus (6). However, no studies of the effect of statolon on murine leukemia and sarcoma virus infection of tissue cultures have been reported.

In the present communication, we describe the effect of Statolon on acute and persistent infection of tissue cultures with murine leukemia and sarcoma viruses. Statolon inhibits not only the growth of murine leukemia virus, but also the *in vitro* cellular transformation induced by murine sarcoma viruses.

Materials and Methods. Viruses. The tissue culture grown murine leukemia and the Moloney strain of murine sarcoma virus (M-MSV) used were furnished by Dr. J. W. Hartley. These had been serially passed by Hartley in Swiss NIH mouse embryo tissue culture (NIH-METC) (7) and were passed by us several times further in the same type of tissue culture prior to the studies described below. MSV pseudotypes (8) were made into virus stock as Moloney procedure concentrates (9) of mouse tumors.

Vesicular stomatitis virus (VSV) was grown in mouse L cell monolayer cultures. Reovirus type 3 (Dearing) was grown in a line of African green monkey kidney cells (Vero) (10).

Cell cultures. Primary cultures of NIH-METC were prepared as previously described (7). Growth medium was 10% fetal bovine serum in Eagle's minimum essential medium (10% FBS-EMEM) with 2 m*M* glutamine, 100 units of penicillin, and 100 μg of streptomycin/ml. This medium was also used for maintenance of cultures used for production of leukemia virus, and for complement-fixing (CF) antigen pools.

The Vero cells were subcultured and maintained in medium 199 supplemented with 5% fetal bovine serum (10). A continuous line of Rauscher leukemia virus-infected rat embryo cells (11) was also used in this study.

Antigen and virus pools. To prepare CF antigen grown in NIH-METC, 14- to 21-day-