# Reports

## Isolation of an Unidentified Agent from the Respiratory Tract of Chickens

*Abstract.* A relatively stable filterable virus, not previously described, which hemagglutinated red blood cells of chickens, was isolated from the tracheae of birds suffering from an unusually severe outbreak of infectious laryngotracheitis. The latter disease concealed the infection caused by the new hemagglutinating agent, which was shown to cause, by itself, a mild and transient illness in susceptible chickens. Preliminary studies indicate that the new agent may be a new entity of the myxovirus group.

A respiratory disease tentatively diagnosed as infectious laryngotracheitis developed in a flock of 3-week-old chickens in southern California. Because the rate of spread and the mortality were unusually high, the case was referred to the University of California Agricultural Experiment Station laboratory at Davis for confirmation. The tracheae of affected chickens were moderately congested and contained petechial hemorrhages in the mucosa, and there were considerable amounts of caseous exudate in the larynx and in the lumen of the upper one-third of the trachea.

Chickens inoculated intranasally with antibiotic-treated or untreated suspensions of the tracheal exudates exhibited depression, dyspnea, rales, a serous nasal exudate, edema and congestion of the larynx, and hemorrhagic inflammation of the tracheae, beginning on the second day. The disease progressed rapidly and was followed by recovery or death by the 15th day. Chickens that recovered were immune to challenge with a virulent laryngotracheitis virus 2 to 6 weeks later and susceptible when challenged intramuscularly with a virulent culture of Newcastle disease virus. Sera of birds that recovered did not contain agglutinins for *Mycoplasma gallisepticum*, hemagglutinating-inhibition or neutralizing antibodies for Newcastle disease virus, or neutralizing antibodies for infectious bronchitis virus.

Tracheal exudates treated with antibiotics and inoculated on the chorioallantoic membrane did not kill embryos during a 6-day incubation period, but the membranes presented raised circular foci indistinguishable from those produced by laryngotracheitis virus; inoculation of chickens, by the intratracheal or intrabursal routes, with the infected membranes produced the typical disease reactions of laryngotracheitis. Inoculation of the tracheal exudates by the allantoic-sac route did not result in mortality in the first two passages, but the allantoic and amniotic fluids of the surviving embryos agglutinated chicken erythrocytes. The fluids of the second, third, and fourth passages hemagglutinated chicken erythrocytes in dilutions of 1:80, 1:160, and 1:320, respectively. A sporadic embryo mortality on the 4th or 5th day began with the third serial passage in embryos inoculated by either the allantoic-sac or chorio-allantoic-membrane routes. These preliminary studies indicated that the infectious exudates contained laryngotracheitis virus and a hemagglutinating agent that appeared to be serologically and immunologically distinct from Newcastle disease virus.

The allantoic and amniotic fluids of the third serial passage through embryos treated with chicken anti-Newcastle disease hyperimmune serum did not neutralize the hemagglutinating agent. The hemagglutinating agent could not be eliminated from the fluids of three additional serial passages, each of which was treated with known anti-Newcastle disease chicken sera. Chickens inoculated intramuscularly with the fluids of the last passage were asymptomatic for 15 days; sera collected from the chickens 3 weeks later inhibited hemagglutination of erythrocytes by the hemagglutinating agent but did not contain hemagglutinins or neutralizing antibodies for Newcastle disease virus. All of the birds were suspectible to challenge intramuscularly with a virulent strain of Newcastle disease virus.

Infected allantoic and amniotic fluids harvested from embryos inoculated in the first passage were treated with anti-Newcastle disease chicken serum, mixed with an equal volume of chicken anti-laryngotracheitis hyperimmune serum, incubated for 3 hours at 4°C, and inoculated into embryonating eggs on the chorioallantoic membrane. Hemagglutinins in the absence of lesions resembling laryngotracheitis, or death of embryos of the fourth, fifth, sixth, and seventh passages, were found in all embryos after a 5-day incubation period.

High mortality began with the eighth serial passage and increased to a proportion of seven of eight embryos inoculated in the tenth serial passage. A pool of the allantoic and amniotic fluids of the tenth passage was divided into aliquots and stored in sealed ampules for further study. Allantoic and amniotic fluids of the living embryos, as well as of embryos that died during the fourth through the tenth passages, consistently presented hemagglutinins and showed an infectivity titer of $5 \times 10^8$ per 0.2 ml of inoculum. Repeated infectivity and cross-neutralization tests in chickens and embryos proved that the laryngotracheitis virus was neutralized and eliminated from the infectious material.

The infectious agent in the allantoic and amniotic fluids of embryos of the tenth passage—fluids from which the laryngotracheitis virus was eliminated —repeatedly passed through the Seitz pad. Filtration of the fluids through graded gradacol membranes showed that the hemagglutinating agent passed through membranes with an aperture diameter of 235 $m\mu$ but not through membranes with an aperture of 140 $m\mu$; this range characterizes the myxovirus group. The hemagglutinating agent was not destroyed by heating infected allantoic and amniotic fluids at 56°C for 30, 60, 90, or 120 minutes.

Chickens 37 days old inoculated intramuscularly or intrabursally with the hemagglutinating agent showed no clinical symptoms during a 3-week observation period. Intratracheal inoculation produced mild rales, which occurred on the 4th to the 6th day in most of the chickens. The agent was readily recovered from the trachea 96 hours after inoculation. Sera of the chickens bled 25 days later contained hemagglutination-inhibition antibodies for the hemagglutinating agent but no inhibition anti-

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

bodies for Newcastle disease virus. The birds showed susceptibility to a challenge dose of virulent Newcastle disease and laryngotracheitis viruses on the 28th day. Eight serial passages of lung suspensions of mice inoculated with the hemagglutinating agent by insufflation did not produce evidence of pneumonia or disease.

The hemagglutinating agent was readily propagated in monolayers of HeLa and pig kidney cells. A mild cytopathogenicity was detected by the 48th hour. Hemadsorption of guinea pig erythrocytes, as described below, was observed by the 48th hour without macroscopic changes in the cell sheets, but adsorption was more pronounced after the 144th hour of incubation.

Immune and hyperimmune sera (*1*) to related viruses were tested for neutralization of the hemagglutinating agent grown in embryonating eggs. Serial tenfold dilutions of the agent were mixed with an equal volume of a heat-inactivated (56°C for 30 minutes) serum diluted 1:10 in tryptose broth and held 1 hour at room temperature. Each mixture was inoculated into each of three 9-day-old embryonating chicken eggs, which were incubated at 35°C for a maximum of 6 days. Infectivity end points were determined by mortality after the 24th hour and the presence of hemagglutinins in the fluids of the surviving embryos. No neutralization of the hemagglutinating agent was demonstrated by antisera of influenza types A and B, mumps, fowl plague (strain Brescia and strain "N"), or Newcastle disease, laryngotracheitis, cello, or Crawley viruses. It was interesting to note that hyperimmune sera prepared with the fluids infected with the hemagglutinating agent in rabbits and chickens did not neutralize the homologous agent.

The hemadsorption test described by Vogel and Shelokov (*2*) and Shelokov *et al.* (*3*) was applied to tissue cultures of HeLa cells infected with the Montana strain of Newscastle disease virus and the hemagglutinating agent. After incubation at 37°C for 144 hours, 0.2 ml of a 0.4 percent guinea pig erythrocyte suspension was added to the washed infected cell sheet. Both agents hemadsorbed chicken, horse, bovine, and guinea pig erythrocytes. However, erythrocytes from guinea pig blood were adsorbed more heavily than those from the blood of the other species.

The hemadsorption-inhibition test showed that hyperimmune sera to the hemagglutinating agent and to Newcastle disease virus specifically inhibited hemadsorption of the homologous virus. None of the sera used in the chicken embryo neutralization tests described above inhibited hemadsorption of the new agent in tissue culture. In addition, parainfluenza 1 and 2 and infectious bronchitis virus immune sera failed to show evidence of inhibition of hemadsorption of guinea pig erythrocytes to the infected tissue culture cells (*3*).

R. A. BANKOWSKI
R. E. CORSTVET
*School of Veterinary Medicine, University of California, Davis*
G. T. CLARK
*Yucaipa, California*

**References and Notes**

1. Antisera used in this study were obtained through the courtesy of several investigators as follows: influenza A and B and mumps, from Dr. E. Lennette, California Public Health Laboratory, Berkeley; fowl plague, strains Brescia and "N," from Dr. J. E. Williams, Animal Disease Eradication Division, Agricultural Research Service, Washington, D.C.; Crawley virus, from Dr. J. F. Crawley, Connaught Medical Research Laboratories, University of Toronto; *Mycoplasma gallisepticum*, from Dr. H. E. Adler, University of California, Davis; and cello, from Dr. V. J. Yates, University of Rhode Island, Kingston. Antisera of parainfluenza 1 and 2 were purchased from Microbiological Associates, Bethesda, Md. All other antisera were prepared by us.
2. J. Vogel and A. Shelokov, *Science* **126**, 358 (1957).
3. A. Shelokov, J. E. Vogel, L. Chi, *Proc. Soc. Exptl. Biol. Med.* **97**, 802 (1958).
4. This investigation was supported in part by grant E-445 of the National Institutes of Health, Bethesda, Maryland.

2 June 1960

## Age Determination by X-ray Fluorescence Rubidium-Strontium Ratio Measurement in Lepidolite

*Abstract.* X-ray fluorescence analysis of several lepidolites whose rubidium and strontium concentrations had already been determined by neutron activation and stable isotope dilution, or both, indicates that this technique can be used for rapid nondestructive reconnaissance rubidium-strontium studies, and that an x-ray analysis method comparable in accuracy to isotope dilution can probably be developed for dating Precambrian lepidolites, as the simple technique presently used has many obvious possibilities for improvement.

Strontium in specimens of the mineral lepidolite has been shown to consist, in general, almost completely of the radiogenic isotope strontium-87, formed through the transmutation of radioactive rubidium-87 (*1*). Thus it is possible to determine the geological "ages" of lepidolites by determining total-Rb/total-Sr ratios alone, without isotopic analysis of the strontium.

Rubidium forms no minerals of its own, proxying for potassium in crystal structures; generally, the K/Rb ratio is in the range 200 to 300 (*2*). However, Rb is enriched in pegmatites, and K minerals in this environment may contain the element as a major constituent. The pink to purple lithium-bearing mica lepidolite, $([K,Rb]Li[Al(OH,F)_2]-Al(SiO_3)_3)$, can contain up to 4 percent Rb, and, in contrast, contains less than 500 parts of Sr per million, the amount depending on age. Hence the analytical problem for lepidolite age determination consists in accurately measuring Rb/Sr ratios ranging from about 90 to above 2500.

L. H. Ahrens *et al.* (*3*) attempted to determine lepidolite age ratios by optical spectrography, but were unable to achieve sufficient accuracy for Rb to make the method generally useful. Recently, however, several groups of workers (*4, 5*) have achieved ratio determinations accurate to 5 percent or better by using the stable-isotope dilution technique, in which Rb and Sr concentrations are measured mass spectrometrically; and analysts at the Harwell installation have shown (*6*) that comparable accuracy can also be achieved by neutron activation analysis.

Stable isotope dilution analysis involves chemical and ion-exchange element separation, preparation of calibrated tracer isotope solutions, and the use of a mass spectrometer, while activation analysis requires the availability of a neutron source of high intensity, a radiochemical laboratory, and counting equipment. Both techniques are difficult and costly. Thus, there is a need for a rapid, inexpensive method of measuring Rb/Sr ratios, if only to make it easier to select samples worth studying by the other techniques.

X-ray fluorescence spectroscopy appeared to merit consideration for use in this application, because it is a rapid, nondestructive technique; hence, the present investigation was begun. A suite of samples whose Rb and Sr contents had previously been determined by isotope dilution and activation was available (*7*). Thus the Rb/Sr ratios determined for these "standards" by x-ray fluorescence could be compared against the ratios previously determined for them by the two established methods.

The x-ray spectrometer used was a General Electric XRD-5. Samples were bombarded by 50 kv CuK$\alpha$ radiation at 50 ma. A LiF crystal followed by a 0.010 inch Soller slit was used for spectrum analysis. The detection system consisted of an argon-methane gas-flow proportional counter run at 1.825 kv, and a Berkeley scaler. Samples were ground to a fine flour in a small steel ball mill which had been precontaminated, and packed into aluminum holders for analysis. The samples were thick compared to the depth of pene-