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Complement-Fixation Test with Tissue-Culture-Antigens as Aid in Recognizing Latent Avian Psittacosis (Ornithosis).

K. F. MEYER, B. EDDIE AND H. YANAMURA.

From the George Williams Hooper Foundation, University of California, San Francisco, California.

The importation of tropical psittacine birds from South America and Australia is always connected with considerable risk in bringing psittacosis to zoölogical gardens, pet stores, aviaries, and breeding establishments of fanciers[1, 2, 3] since latent infections, which may relapse or become activated in transit, are fairly common. As a rule, the existence of psittacosis in a shipment is established by autopsies and the inoculation of mice with the spleens of the few birds which succumb while held in quarantine. Present regulations permit the prompt release of all birds of a consignment provided no deaths are noted in the flock for 2 weeks. On the other hand, it has been fully appreciated that apparently healthy birds may be active or potential shedders of virus. Under the circumstances, it has recently become customary to destroy the entire shipment. Valuable and expensive birds, which on autopsy proved to be free from psittacosis, were thus sacrificed unnecessarily since no methods had been available to segregate the infected from the non-infected. Experiments recently conducted on a shipment of Australian parrots indicate that the complement-fixation test may detect carrier birds with a high degree of certainty.

The complement-fixation tests for psittacosis, originally introduced by Bedson[4] and proven invaluable in the early diagnosis of human infections has been adopted with certain modifications for the examination of parrots. As antigens, concentrated and trypsin-digested cultures of the psittacosis virus in Rivers-Li media have been employed. The preparation of these antigens is briefly as follows: To a medium consisting of 42.5 cc of Tyrode solution and 2.5 cc of chick embryonic tissue fragments (5 cc Tyrode solution to each 11th-day decapitated chick embryo) are added 5 cc of passage-culture, held in 250 cc Erlenmeyer flasks, cotton-plugged and sealed during incubation with "parafilm". After 3 days' incubation, the culture reveals

1 Levinthal, W., *Lancet*, 1935, May 25, 1207.

2 Morgan, M. T., *Bull. Off. Inter. d'Hygiene Publique*, 1938, **30**, 1252.

3 Troup, A. G., Adam, R., and Bedson, S. P., *Brit. Med. J.*, 1939, **1**, 51.

4 Bedson, S. P., *Lancet*, 1935, **2**, 1277; 1937, **2**, 1477.

TABLE I.
Complement-Fixations with Sera of Infected and Healthy Australian Parrots.

No.	History of parrot	Virus demonstrated	Complement fixation reaction: Tissue culture Australian virus	Tissue culture California virus	Tissue culture control
1	*Kakatoe galerita*	0 I	1:16++++; 1:16++++	1:8++++; 1:4++	0
2	*Kakatoe galerita*	Spleen	>1:32; 1:32++++	>1:32++++; 1:32	0
4	*Kakatoe galerita*	Spleen	1:64++++; 1:64+++ 1:128+++; 1:128+	1:64++++ 1:128+	0
5	*Kakatoe galerita*	0 I	>1:64++++	>1:64++++	0
6	*Kakatoe galerita*	Spleen	>1:128; >1:128++++	>1:128++++	0
7	*Kakatoe sanguinea*	Spleen	1:16++++; 1:16+++ 1:64++; 1:64±	1:32++++; 1:32+++ 1:64+++	0
11	*Kakatoe roseicapilla*	0	0	0	0
14	*Kakatoe roseicapilla*	0 I	1:16±; 1:10++	1:16++; 1:10++	0
15	*Kakatoe roseicapilla*	0	0	0	0
17	*Kakatoe roseicapilla*	0	0	0	0
20	*Nymphicus hollandicus*	0	1:8+++; 1:10++	1:32++++; 1:10++	0
24	*Nymphicus hollandicus*	Spleen and liver	1:64++++; 1:16++++	1:64++++; 1:32++++	0
29	*Kakatoe sanguinea*	0	0	0	0
30	*Kakatoe roseicapilla*	0	1:8+++; 1:2++	1:8++++	0
31	*Platycercus elegans*	Spleen	1:16++++; 1:16±	>1:32++++; 1:32++++	0

an abundance of elementary (L.C.L.) bodies; only cultures rich in virus should be used. It is shaken mechanically for 15 minutes; centrifuged in horizontal position for 15 minutes at 3,000 r.p.m.; the supernatant fluid is then spun in an angle centrifuge for 1½ hours at 4,000 r.p.m. The sediment is suspended in buffered physiologic salt solution (McIlvaine M/50) and steamed for 30 minutes. An antigen with greater binding power may be obtained by digesting the sediment from the angle centrifugation after which thorough washing will yield a suspension rich in virus particles. They are, as a rule, suspended in 2/5 of the original volume of buffered salt solution (M/50, pH 7.6) and steamed for 30 minutes.

The sera are obtained by bleeding the ether-anesthetized parrots from the ulnar vein *(V. cutanea ulnaria)* of the wings. To prevent premature clotting, it is advisable to cover the disinfected skin area over the vein with a few drops of 1% heparin solution and to use dry (20 gauge 1½ inches long) needles and syringes. The sera inactivated at 56° for 30 minutes are diluted with saline as required; then 0.25 cc of each is placed in sterile tubes, 0.25 cc of antigen added, then mixed with 0.1 cc of complement (titrated and diluted to contain 2 hemolytic units in 0.1 cc) and 0.4 cc of saline. The tubes are shaken and incubated in a waterbath at 37°C for 2 hours; 0.5 cc of sensitized cells (2 units per 0.5 cc of 2% suspension) are then added and the mixture is again incubated for 1 hour. Readings are recorded immediately after removal from the waterbath and after 12 hours in the icebox. It is important to titrate a serum against 3 to 4 antigens (infected, non-infected cultures of American and Australian virus strains), thus at least 48 tubes are required for the evaluation of one serum.

A recent shipment of Australian kakatoes and parrots to California offered an opportunity to test the accuracy of the complement-fixations. In quarantine, 10 of 14 parrots, which died in an emaciated state, proved to be infected with the virus of psittacosis. The remaining 31 birds were bled, then carefully autopsied and the spleen and liver tested on mice. The sera were subjected to the delicate complement-fixation test with the results shown in Table I.

The sera of the latent-infected parrots gave strong permanent and specific reactions both with Australian and American virus culture antigens. Birds free from virus either entirely failed to react to the heated antigens, gave complete fixation of 2 M.H.D. of complement in dilutions of the sera 1:1 to 1:64 or the fixation recorded immediately after removal from the waterbath faded after 12 hours in the icebox. In the light of previous experiences with the sera of

mice, guinea pigs, and other animals which had been infected or immunized, it is believed that the fixation-reactions of the sera of virus-free parrots are indicative of a past infection. It must be reserved for future studies to decide if these reactions are also indicative of immunity. The complement-fixation test in its present state does not distinguish an infection from a sterile immunity. From the standpoint of public health, parrots which specifically react in the complement-fixation must be destroyed.

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Effects of Fast Neutrons on Chromosomes in Mitosis.*

A. MARSHAK.† (Introduced by John H. Lawrence.)

From the Radiation Laboratory, University of California, Berkeley, Calif.

The neutron source was a beryllium target bombarded by deuterons accelerated to 8 million volts in the cyclotron of Lawrence and Cooksey.[1] The neutrons so obtained were collimated in a beam as described by Aebersold.[2] The biological material to be treated was placed just outside the 10 x 10 cm port at the end of the collimation apparatus at a distance of 70 cm from the target. Ionization produced by the neutrons was measured in arbitrary "n" units, and "n" unit being that amount of ionization produced by neutrons which gives the same reading on a 100 r Victoreen thimble ionization chamber as does one roentgen of X-rays.

Six-day-old seedlings of *Vicia faba* and *Pisum sativum* were mounted on an annular wooden holder and oriented so that the root tips lay in the center of the 3-inch aperture which was covered on either side by a sheet of wet filter paper and a sheet of celluloid 5.4 thousandths of an inch thick. The cotyledons and epicotyl of the seedlings lay outside the neutron beam, so that corrections for scattering may be neglected. The much smaller seedlings of *Solanum lycopersicum* were mounted between 4 sheets of wet filter paper 3½ inches in diameter lying between 2 sheets of cellophane held on a round wooden embroidery hoop 6 inches in diameter. All seedlings

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† Fellow of the John Simon Guggenheim Memorial Foundation.

1 Lawrence, E. O., and Cooksey, D., *Phys. Rev.*, 1936, **50**, 1131.

2 Aebersold, P. C., *Phys. Rev.*, 1939, in press.