

an indication of an initial stress reaction of the animal to ionizing radiations.

Storer(7) found that the testes weight of rats exposed to 500 r total body X-rays was decreased to approximately 74% of the control weights 4 weeks after irradiation. In both experimental groups reported here the gonadotropin activity of the pituitary at 4 weeks was not significantly different from the controls. It seems therefore that the testes damage observed 30 days after irradiation may be due to damage *in situ* and not the result of any continued alteration of gonadotropin activity within the pituitary. Neither can one exclusively reconcile the gonadotropin activity with histologic changes in the pituitary (e.g. castration cells) for in all probability the cellular changes observed are the result of a severe imbalance of several endocrine principles.

Summary. Rabbit pituitaries from animals exposed to 750 and 1100 r of total body Cobalt-60 gamma irradiation showed an early

suppression of gonadotropin principles as measured by the chick gonad response. The 750 r group showed the depression on the 8th day after irradiation while in the 1100 r group the suppression was evident by day one and was sustained through day 7. After this time the gonadotropin activity in both groups returned to the normal level.

1. Botkin, A. L., Praytor, E. H., Austing, M. E., and Jensen, H., *Endocrinology*, 1952, v50, 550.
2. Monroe, R. A., Rust, J. H., and Trum, B. F., *Science*, 1954, v119, 65.
3. Patt, H. M., Swift, M. N., Tyree, E. B., and John E. S., *Am. J. Physiol.*, 1947, v150, 480.
4. Witschi, E., Levene, W. T., and Hill, R. T., *Proc. Soc. Exp. Biol. and Med.*, 1932, v29, 1024.
5. Wilding, J. L., Simons, C. S., and Rust, J. H., *Nucleonics*, 1952, v10, 36.
6. Lane, J. J., and Paysinger, J. R., *Proc. Soc. Exp. Biol. and Med.*, 1953, v84, 396.
7. Storer, J. B., and Sanders, P., *ibid.*, 1953, v83, 259.

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A Hemagglutination Inhibition Test for Infectious Sinusitis of Turkeys.* (21005)

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Infectious sinusitis (IS) of turkeys is prevalent and of considerable economic importance in the United States and Canada. The cause of this disease is now generally considered to be a pleuropneumonia-like organism (PPLO) (1,2). Egg transmission of the infectious sinusitis agent has been shown to occur and this means of spread appears to play a major role in the dissemination of the disease(2-5). Efforts to devise a serological test, employing *in ovo* neutralization and cold hemagglutination, for use in the study of this disease, have not succeeded(6).

It has been shown that PPLO derived from chickens cause agglutination of chicken red

blood cells and that sera of chickens infected with these organisms possess the ability of inhibiting this reaction(7,8). In this paper are presented the results of testing the sera of 4 turkey flocks for the presence of hemagglutination inhibition (HI) antibodies.

Materials and methods. The methods of isolation of the PPLO used in this study are essentially the same as outlined for the isolation of PPLO from chickens(9), except that the organisms were isolated from sinus exudate of infected turkeys. The blood samples were obtained at commercial killing plants in Ontario from turkeys which were from 26-50 weeks of age. In principle the test was performed in the same way as the HI test for Newcastle disease(10). The technic of performing this test has been described in detail

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TABLE I. Hemagglutination Inhibition Antibodies in Turkey Sera.

Flock	No. of turkeys ×100	Age, wk	Breed	No. of sera tested	HI titers*										% posi- tive sera	Clinical disease†	
					<5	5	10	20	40	80	160	320	640	% Sinusitis		Respiratory symptoms	
L	30	50	WH	144	13	23	54	31	15	6	2			90	0	Marked	
	180	32-35	BBB	126	2	1	5	9	46	50	7	4	2	98.4	60	"	
D	18	30	Neb.	33	3	0	3	9	5	10	2	1		90.9	25	Slight	
C	16	30	BBB	38	38									0	<1	None	
W	21	26	BBB	73	73									0	0	"	

* Expressed as reciprocals of highest serum dilution causing complete inhibition of hemagglutination. (<5 = negative.)

† Based on observations during entire growing period.

WH = White Holland, BBB = Broad Breasted Bronze, Neb. = Nebraskan.

previously(11). A single strain (isolated from flock D) was used to conduct these tests.

Results. The results of testing the sera of 414 turkeys from 4 flocks are presented in Table I. The titers reached in the positive flocks were in the range of 5-80, but sera with titers up to 640 were found.

The incidence of sinusitis in the flock is an estimate based on observations in the killing plant and in the field. Sinusitis was first noticed in flock L when the BBB birds were 8 weeks of age. Although the WH birds in the flock were hatched and raised in the same buildings as the BBB birds no sinusitis developed in this group. However, the WH poults, in common with all other birds on the premises, did develop a severe respiratory infection around 12 weeks of age. The respiratory symptoms persisted for 5-6 weeks and caused considerable unthriftiness.

The incidence of sinusitis in flock D was determined 4 weeks prior to killing, since at this time the author visited the flock and injected each infected bird with streptomycin. An occasional bird with sinusitis was seen in flock C but this developed when the birds were around 20 weeks of age and the failure to detect any positive sera may be due either to the small sample size and/or to the fact that none of the birds which were bled showed any evidence of sinusitis. No sinusitis was observed in flock W at any time during the life of the birds. Prior to the choice of strain D as the test strain, tests were performed to determine if the avian PPLO were antigenically homogeneous with respect to the HI test. A strain of PPLO isolated from flock L was studied in order to compare the sera of flocks L and D with homologous and heterologous antigens. Two other strains obtained from chickens were used to extend those observations. Tests on 15 sera from each flock indicated that if a serum were positive it would have the same titer independent of the PPLO strain used for antigen. Conversely, sera showing no HI antibodies with one strain were negative with the other 3 strains tested. In the sera obtained from flock L it was noticed that an inhibitory substance was present in 9 of the 126 BBB sera tested. This substance prevented the inhibition of agglu-

tion by positive sera at the $\frac{1}{5}$ dilution. However, it did not interfere with the interpretation of the tests and could not be destroyed by heating at 56°C for $\frac{1}{2}$ hour. No such interference was observed with the sera from flock D or with the WH sera from flock L.

The reproducibility of the test over varying periods of time was examined. Sera from flock L (BBB) were tested when strain D was at the 6th broth passage and again when the strain was at the 15th passage, or 8 weeks later. The serum titers on both occasions showed almost perfect agreement except for a few sera where the titer dropped from 160 or 320 to 80. This may be due to deterioration of antibody over the period of storage at cold room temperature. A frozen pooled sample was used as a positive control and was tested at each passage level from the 6th to 15th. The titer of this standard serum was usually 40, but occasionally 80. Such variation is inherent in the test.

Discussion. This study was initiated with the hope that a diagnostic test could be devised which would be of practical value in the selection of PPLO-free breeding stock. The results presented indicate that a hemagglutination inhibition test might serve this purpose. Although testing large numbers of sera from breeder flocks would be tedious, the advantages of obtaining disease-free replacement stock would more than repay this effort. It would appear that destroying those birds showing sinusitis does not serve to eliminate the disease from a flock since a large number of birds have been found with PPLO antibodies, but no evidence of sinusitis. Although the WH birds in flock L did not develop sinusitis, they were nevertheless affected with

a chronic respiratory condition. The serological results indicate that PPLO were involved in both these conditions. The factors governing development of sinusitis in a bird infected with PPLO are obscure, but it is apparent that infection with PPLO does not necessarily result in sinusitis.

Summary. 1. Four hundred and fourteen sera from 4 turkey flocks have been tested by means of hemagglutination inhibition test utilizing a PPLO antigen. 2. This test gives reproducible results and gives promise of being useful in screening breeder flocks. 3. PPLO isolated from chickens and turkeys are apparently antigenically homogeneous with respect to this HI test as based upon 4 strains isolated from widely separated sources.

1. Markham, F. S., and Wong, S. C., *Poultry Sci.*, 1952, v31, 902.
2. Grumbles, L. C., Phillips, E., Boney, W. A., and Delaplane, J. P., *Southwestern Vet.*, 1953, v6, 166.
3. Jerstad, A. C., Hamilton, C. M., and Smith, V. E., *Vet. Med.*, 1949, v44, 272.
4. Jerstad, A. C., and Hamilton, C. M., *Poultry Sci.*, 1948, v27, 802.
5. Grumbles, L. C., and Boney, W. A., *Proc. U. S. Livestock Sanit. Assn.* 54th Ann. Meet., 1950, 166.
6. Prier, J. E., and Dart, C., *Cornell Vet.*, 1948, v38, 208.
7. Van Herick, W., and Eaton, M. D., *J. Bact.*, 1945, v50, 47.
8. Jacobs, R. E., Jungherr, E., Luginbuhl, R. E., and Gianforte, E., *Proc. 25th Ann. Meet. Lab. Workers Pullorum Dis. Control*, Amherst, Mass., 1953.
9. Fahey, J. E., and Crawley, J. F., *Canad. J. Comp. Med.*, 1954, in press.
10. Crawley, J. F., *ibid.*, 1954, in press.
11. Fahey, J. E., and Crawley, J. F., *ibid.*, 1954, in press.

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