glucuronidase was preferentially discharged during incubation with starch granules.

1. Lichtenstein, L. M., Osler, A. G., J. Exp. Med., 1964, v120, 507.
2. Pruzansky, J. J., Patterson, R., J. Allergy, in press.
3. ———, J. Immunol., accepted for publication.
4. Sheldon, J. M., Lovell, R. G., Matthews, K. P., A Manual of Clinical Allergy, W. B., Saunders, Philadelphia, Pa. 1953.
5. Shore, P. A., Burkhalter, A., Cohn, V. H., Jr., J. Pharmacol. Exp. Therap., 1959, v127, 182.
6. Hirschhorn, R., Weissman, G., Proc. Soc. Exp. Biol. and Med., 1965, v119, 36.
7. Mota, I., J. Physiol., 1959, v147, 425.
8. Graham, H. T., Lowry, O. H., Wheelwright, F., Lenz, M. A., Parish, H. H., Blood, 1955, v10, 467.
9. Austen, K. F., Humphrey, J. H., Adv. Immunol., 1963, v3, 1.

Received August 20, 1966. P.S.E.B.M., 1967, v124.

Transmission of Marek's Disease with Oral Washings and Feces From Infected Chickens. (31666)

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Marek's disease (MD) is a disease of the avian leukosis complex that causes severe mortality and economic loss in young chickens. The etiological agent of MD is unrelated to viruses causing lymphoid leukosis, another major disease of the avian leukosis complex (1). Also, MD has been observed in chickens known to be free of lymphoid leukosis virus infection(2).

The modes of natural transmission of MD are poorly documented. Under natural and experimental conditions the disease appears highly contagious and Sevoian(3) has shown the agent to be airborne. Certain beetles may be carriers of the infectious agent and can transmit the disease when eaten by susceptible chickens(4). Early attempts at transmission with feces or oral washings were apparently successful in some cases(5,6,7) but were negative in others(8,9). Since horizontal transmission by both direct and indirect contact appeared to be highly important in the epizootiology of MD, studies were undertaken to explain further the mechanisms involved. This preliminary report describes the presence of the infectious agent in oral washings and feces from chickens experimentally infected with the JM strain of MD.

Materials and methods. Marek's disease agent. The JM strain of MD, isolated by Sevoian *et al*(10), was used in this trial. This strain has been maintained at this laboratory by serial passage in chickens at 4- to 6-week intervals with heparinized whole blood or tumor suspension. No change in potency or pathologic characteristics has occurred with passage.

Chickens. White Leghorn chickens of line 7, an inbred line developed at this laboratory, were used in these studies. Line 7 chickens are highly susceptible to MD but are genetically resistant to infection with common strains of lymphoid leukosis viruses(11).

Inocula. Oral swabs were obtained by massaging the oral cavity of each donor bird with sterile cotton swabs. Since 5 or 6 such swabs were prepared from each donor, an attempt was made to insure that each swab received approximately equal quantities of fluid and mucus. The swabs immediately were placed in individual sterile glass tubes.

Oral washings were collected 2 hours after swabs were taken to permit the oral fluids to be replenished. The oral and nasal cavities of each donor bird were repeatedly irrigated with 5 ml of cell culture media consisting of medium 199 with 2% bovine fetal serum, 10% tryptose phosphate broth and penicillin and streptomycin at 10,000 units and 10 mg per ml respectively. The washings were pooled and centrifuged at 4 C for 5 minutes at 400 $\times$ *g*. About 7/8 of the supernatant fluid was carefully drawn off and used for inoculation.

TABLE I. Marek's Disease Induction by Inoculation of Line 7 Chickens with Oral Washings, Fecal Extracts and Blood from Donor Chickens with JM-Induced Marek's Disease.

Inoculum	Dose (ml)	Route*	No. inoculated	Exp No.†	Marek's disease response§ % mortality	% mortality plus positive survivors‡	Median days to death
Oral swab	—	swab	16	9	0	11	—
" washings	.2	ia	16	12	75	100	38
" "	.1	in	16	15	0	0	—
Fecal extract	.2	ia	16	12	83	100	44
" "	.1	in	16	14	7	21	62
Whole blood	.2	ia	16	16	94	100	29
None	—	—	16	7	0	0	—

* Swab = direct application to oral cavity; ia = intra-abdominal; in = intranasal.
† Number inoculated less nonspecific mortality. 24 of the 27 nonspecific deaths due to starvation and dehydration in the first 10 days.
‡ Includes survivors positive by gross lesions or by definite microscopic nerve lesions.
§ Experimental period was 70 days.

Fecal extracts were prepared as follows: the cloaca of each donor chicken was thoroughly swabbed with sterile cotton swabs. Each swab was placed in 3 ml of cell culture media. The samples were gently mixed, pooled, diluted to a total volume of 10 ml with additional media and centrifuged in the manner described for oral washings. Supernates were carefully removed and used for inoculation.

Whole blood from each donor was collected in heparin (20 units per ml of blood), pooled and used undiluted.

All inocula were maintained at about 2 C. in wet ice during the 1- to 2-hour interval between collection and inoculation. The oral washing and fecal extract inocula produced no bacterial growth in tryptose phosphate broth during 3 days incubation at 37 C.

Experimental. Oral swabs, oral washings, fecal extracts and heparinized whole blood inocula were prepared from materials collected and pooled from three 29-day-old chickens representing the 18th passage of the JM strain. The donors were lame or paralyzed and at necropsy showed distinct gross lesions (enlarged nerves, gonadal tumors) of MD. The oral swabs were administered by massaging the oral cavity of day-old recipient chicks, one swab per chick. The oral washings and fecal extracts were inoculated intra-abdominally and instilled intranasally using 0.2 and 0.1 ml per chick respectively. All lots contained 16 one-day-old chicks and were held in modified Horsfall-Bauer isolators for 10 weeks. All mortality and birds killed at termination of the trial were necropsied and examined for gross lesions of Marek's disease. Histological examination of the vagus nerve, brachial and sciatic plexuses, and gonad was made on all birds in which gross lesions were questionable or absent. Birds with nerves containing lymphocytes, plasma cells or reticulum cells in concentrations greater than or equal to that illustrated in Fig. 1 were considered positive for MD. Normal nerves contained none of these cells (Fig. 2).

Results and discussion. Oral washings, fecal extracts and whole blood produced high MD mortality (75 to 94%) when inoculated intra-abdominally (Table I). All survivors of these lots had gross or microscopic lesions. Lesions in birds inoculated with oral washings or fecal extracts were typical of MD (Fig. 3 and 4) and were indistinguishable from those produced by blood inoculation. Since uninoculated control chickens were negative by all response criteria, the presence of MD agent in the oral washings and fecal extracts used in this trial is confirmed.

The disease was induced by fecal extracts given intranasally but the magnitude of response was much lower than when the same material was inoculated intra-abdominally. Oral washings inoculated intranasally gave no response. When oral fluids were transferred with swabs to the oral cavity of recipient chickens, no MD mortality occurred and only

one of 9 survivors had minimal microscopic lesions. It was obvious that transmission of MD was much less efficacious with intranasal inoculation or administration of oral swabs than with intra-abdominal inoculation. However, the 21% response obtained with fecal extracts showed that infection *via* the intranasal route was possible.

The comparative potency of oral washings and fecal extracts, when estimated from median days to death (MDD), was considerably less than that of whole blood. However, the MDD response for chickens inoculated with oral washings or fecal extracts was similar to values obtained in other experiments when line 7 chickens were exposed by direct contact with

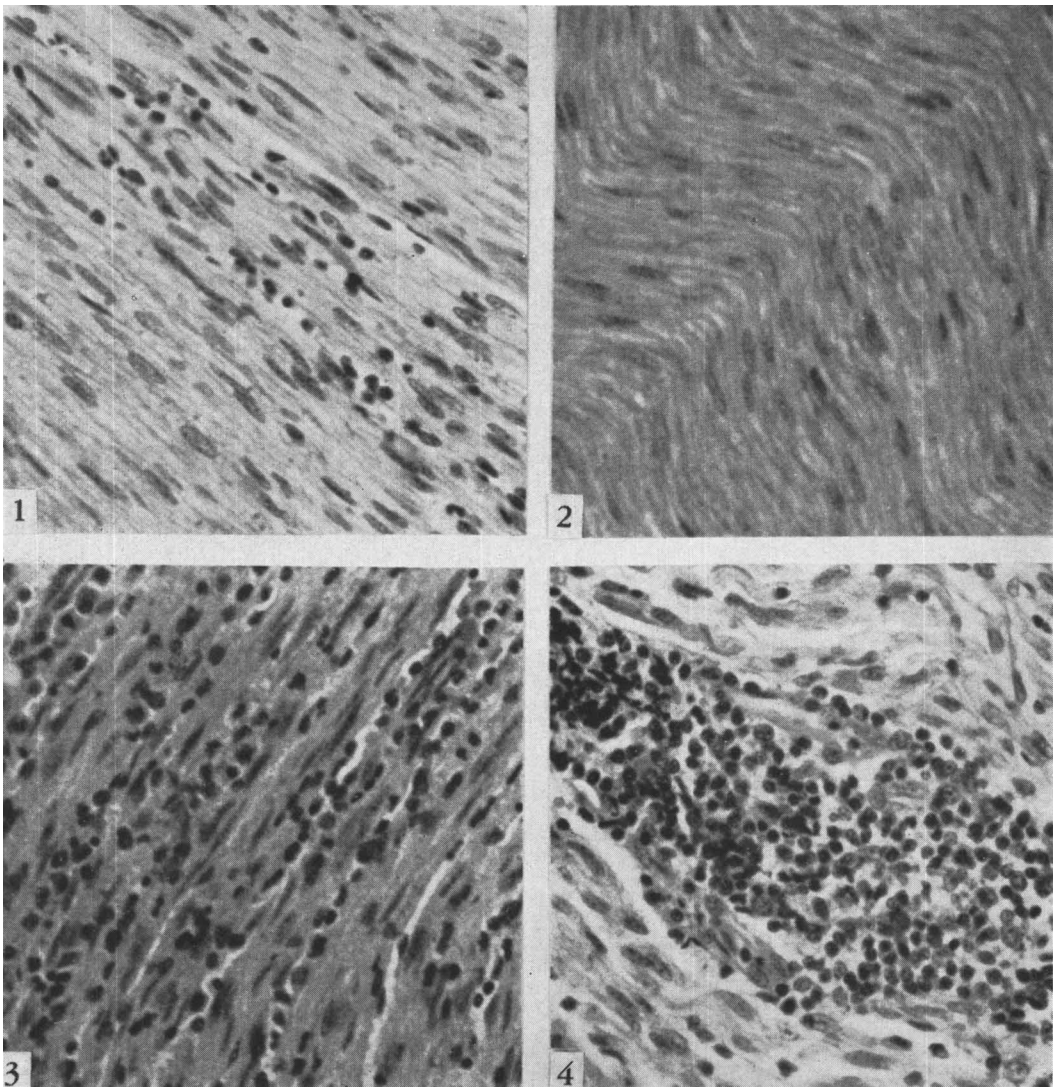

FIG. 1. Vagus nerve from a 37-day-old chicken showing scattered lymphocytes. Nerves with lesions of equal or greater severity were considered positive for Marek's disease. (× 490).

FIG. 2. Normal vagus nerve from a 70-day-old chicken. (× 490).

FIG. 3. Nerve from a chicken inoculated intra-abdominally with fecal extracts and dead at 37 days from Marek's disease showing an extensive, diffuse accumulation of pleomorphic lymphocytes. (× 490).

FIG. 4. Nerve from a chicken inoculated intra-abdominally with oral washings and killed at 70 days with gross Marek's disease lesions showing extensive diffuse and focal accumulations of pleomorphic lymphocytes. (× 490).

JM infected penmates.

The peripheral nerves of some birds considered histologically negative contained a few scattered cells in concentrations intermediate between those in Fig. 1 and 2. Such cells may have constituted minimal MD lesions, however, the lack of established methods for the identification of such lesions justified the use of an arbitrary histologic criterion (Fig. 1) for the diagnosis of all birds. Also, the isolators used in this trial may not have prevented entirely horizontal transmission of MD between lots because the degree of isolation was less than absolute and the MD agent spreads very easily. Consequently, low incidence responses, *e.g.*, the single histologic positive survivor following oral swab inoculation, cannot be definitely attributed to the inoculum. In contrast, all other responses in this trial were of greater magnitude and were considered to be the result of the administered inoculum.

Twenty-four chickens died from starvation and dehydration during the first 10 days of this trial and, although unevenly distributed among lots, did not appear to influence the interpretation of these results.

The presence of MD agent in excretions and secretions of experimentally infected chickens as described above is further supported by results of two trials currently in progress. In one, oral washings and fecal extracts collected from 4-week-old, JM-inoculated chickens induced MD by intra-abdominal inoculation. In this test, the potency of oral washings appeared greater than that of fecal extracts. All uninoculated control birds were negative. In another trial, 100% of isolator-reared chickens developed MD following contact exposure to two sources of litter or droppings from 4- and 6-Week-old, JM-inoculated chickens. Lots receiving autoclaved litter or no treatment were negative.

Burmester(12,13) found virus in the feces and oral washings of chickens with lymphoid leukosis and established the role of such virus in the horizontal transmission of the disease. Although the data reported here confirm the presence of infectivity in oral washings and feces of experimentally infected chickens, the importance of this infectivity in the natural spread of MD is yet to be resolved. However, the possibility that such sources of infectivity may partially account for the high rate or horizontal transmission of MD under natural conditions should be considered.

Summary. Marek's disease was transmitted with oral washings and fecal extracts from 4-week-old, JM-inoculated chickens to day-old chickens by inoculation. All birds inoculated with oral washings or fecal extracts by the intra-abdominal route were positive while intranasal instillation of these materials gave responses of 0% and 21% respectively during a 10-week experimental period. An attempt to transmit Marek's disease by a direct transfer of oral fluids with cotton swabs from donor to recipient chicks was unsuccessful.

1. Biggs, P. M., Payne, L. N., Nat. Cancer Inst., Monograph 17, 1965, 83.

2. Witter, R. L., Calnek, B. W., Levine, P. P., Avian Dis., 1966, v10, 32.

3. Sevoian, M., Chamberlain, D. M., Larose, R. N., ibid., 1963, v7, 102.

4. Edison, C. S., Schmittle, S. C., Goode, R. B., Lal, J. B., Am. J. Vet. Res., 1966, v27, 1053.

5. Seager, E. A., Vet. J., 1933, v89, 454.

6. Jungherr, E., Storrs Agr. Exp. Sta. Bull. No. 218, 1937.

7. Fritzsche, K., Z. Infekt-Krankh. Haustiere., 1938, v52, 51.

8. Thorp, F., Graham, R., Vet. Med., 1936, v31, 82.

9. Barber, C. W., Cornell Vet., 1948, v38, 130.

10. Sevoian, M., Chamberlain, D. M., Counter, F. T., Vet. Med., 1962, v57, 500.

11. Crittenden, L. B., Okazaki, W., Reamer, R. H., Nat. Cancer Inst., Monograph 17, 1964, 161.

12. Burmester, B. R., Gentry, R. F., Poultry Sci., 1954, v33, 836.

13. Burmester, B. R., ibid., 1956, v35, 1089.

Received August 24, 1966. P.S.E.B.M., 1967, v124.