Accelerated Clinical Course of Marek's Disease in Chickens Fed Penicillin (37617)

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This paper reports results obtained in studies in experimentally induced Marek's disease in chickens maintained on antibiotic supplemented diets. The findings show that incorporation of penicillin into the diet was associated with an enhanced clinical course of the disease, whereas incorporation of 5 other antibiotics (except for a limited enhancement associated with use of a streptomycin-supplemented diet) was not.

Materials and Methods. Chickens were white Leghorns (obtained from SPAFAS, Inc., Norwich, CT) that were bred and maintained before but not during experimental use under specific-pathogen-free conditions. The chickens were fed antibiotic-free feed (manufactured according to NIH specifications 11-29 under contract No. 71-770 CC) from the time of hatching until the end of the test periods indicated in Tables I and II, a period which preceded virus exposure by 3 to 5 days.

Antibiotics employed are listed in Table I and were added daily to the drinking water in amounts shown in the table.

Virus and virus administration. The JM strain of Marek's disease virus (MDV) was employed and was maintained by serial intraperitoneal passage of infected chicken blood in 3- to 5-day-old chickens. When the inoculated chickens showed clinical signs of Marek's disease (MD) which was usually 4- to 5-wk postinoculation, they were bled by cardiac puncture. A single pool of heparinized whole blood constituted the test virus suspension and was used within 4 hr of harvest.

Contact infection was accomplished by housing uninoculated test chickens and MDV-infected chickens in the same room. Contact infection was successfully avoided by housing uninoculated test chickens in a separate room (physically distant from MDV infected quarters) in which MDV had not been used.

Clinical, virologic and pathologic evidence of MDV infection. Test and control chickens were examined daily for signs of disease: when these occurred, heparinized blood samples were taken for inoculation into duck embryo cell cultures. Appearance in the inoculated cultures of cytopathic changes pathognomonic for MDV was considered evidence for MDV infection in the blood donor chicken. Necropsy procedures in test and control chickens consisted of gross examination of the vagus and sciatic nerves, the brachial plexus and visceral organs. Presence of nerve enlargement and visceral tumors were considered pathognomonic for MD.

Results. Time of death in chickens on antibiotic-supplemented diet following inoculation of MDV. Observations on day of death following intraperitoneal inoculation of MDV in chickens whose diet was supplemented by addition to the drinking water of one or the other of the 6 antibiotics are presented in Table I. These data show that by postinoculation Day 23 a significant number of deaths (6 of 12) had occurred in chickens on the penicillin-supplemented diet, by the same postinoculation day no deaths had occurred in any of the 19 chickens on the control (antibiotic free) diet and in only 1 (in the Kantrex group) of 56 chickens on diets supplemented with antibiotics other than penicillin. In the interval between postinoculation Days 23 and 30, deaths occurred in all of the antibiotic-supplemented diet groups as well as in the control groups; however, the number of deaths in the penicillin- and streptomycin-supplemented groups was significantly

TABLE I. Time, Number and Percentage of Deaths in MDV Exposed Chickens on Antibiotic Supplemented Diets.[a]

Experimental group	Diet supplement		No. chickens in group	Accumulative deaths on day															
	Antibiotic	Amount[b]		10	23		25		30		35		39		42		46		79	
					No.	%[t]	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
MDV by injection	None	—	19	0	0		0		3	16	5	26	5	26	6	32	17	89	19	100
	Penicillin[c]	250	12	0	6	50	7	58	8	66	10	83	10	83	10	83	10	83	12	100
	Streptomycin[d]	445	12	0	0		0		6	50	9	75	9	75	10	83	10	83	12	100
	Kantrex[e]	200	12	0	1	8	1	8	2	17	5	42	7	58	8	66	8	66	9	75
	Garamycin[f]	25	9	0	0		0		1	11	3	33	4	44	5	56	7	78	9	100
	Achromycin[g]	125	11	0	0		1	9	3	27	4	36	4	36	5	45	5	45	8	73
	Totacillin[h]	125	12	0	0		1	8	2	17	4	33	5	42	7	58	11	92	11	92
MDV by contact	None	—	24	0	0		0		0		1	4	3	12	10	42	22	92	22	92
	Penicillin[c]	250	10	0	0		0		8	80	9	90	9	90	9	90	9	90	9	90
	Streptomycin[d]	445	9	0	0		0		0		0		1	11	2	22	8	89	8	89
	Kantrex[e]	200	10	0	0		0		0		0		5	50	8	80	8	80	9	90
	Garamycin[f]	25	10	0	0		0		0		1	10	2	20	2	20	4	40	6	60
	Achromycin[g]	125	22	0	0		0		0		0		0		0		6	27	16	73
	Totacillin[h]	125	10	0	0		1	10	1	10	2	20	2	20	3	30	3	30	7	70

[a] MDV = Marek's disease virus.
[b] Added daily to drinking water (μg/ml of drinking water).
[c] Potassium penicillin G, Eli Lilly Co.
[d] Streptomycin sulfate, Eli Lilly Co.
[e] Kanamycin sulfate, Bristol Laboratories.
[f] Gentamicin sulfate, Schering Corp.
[g] Achromycin tetracycline HCl, Lederle Laboratories.
[h] Ampicillin trihydrate, Beecham Pharmaceuticals.
[t] % of total number.

higher (8 of 12 and 6 of 12, respectively) than in the control group (3 of 19) or in any of the other antibiotic-supplemented groups (range: 1 of 9 for Garamycin to 3 of 11 for Achromycin). These differences were clearly observable as late as postinoculation Day 42 when 10 of 12 chickens in each of the penicillin and streptomycin groups had died whereas the number of deaths in the control group was 6 of 19 and in the other diet-supplemented group, deaths ranged from a low of 5 of 11 for the Achromycin group to 8 of 12 for the Kantrex group. By postinoculation Day 79 when all of the chickens had died in the control group and in the penicillin and streptomycin groups, there were 7 surviving chickens distributed as follows: 3 of 12, 3 of 11 and 1 of 12 in the Kantrex, Achromycin and Totacillin groups, respectively.

Time of death in chickens on antibiotic-supplemented diet following MDV contact. Table I shows that by postcontact Day 30 a significant number of deaths (8 of 10) had occurred in the penicillin-supplemented group when only one other death (in the Totacillin group) had occurred in 85 chickens among the other groups. This striking difference between the number of deaths in the penicillin group and the other groups was still observable as late as postcontact Day 39 when death had occurred in 9 of 10 chickens in the former group while in the latter groups the incidence of death ranged from a low of 0 of 22 in the Achromycin group to 5 of 10 in the Kantrex group. By Day 79, there was no significant difference in the incidence of death among the groups.

Deaths in chickens on penicillin-supplemented diet in MDV-contaminated and MDV-free environments. All 9 chickens on a penicillin-supplemented diet housed in a room free of MDV remained well for a 60-day observation period, while all 10 chickens on the same penicillin-supplemented diet housed in a room with other chickens with MD clinical signs died of MD during the same test period (Table II).

TABLE II. Deaths in Chickens on Penicillin Supplemented Diet in MDV-Contaminated and MDV-Free Environments.

Exposure			Deaths[b]	
Penicillin[a]	MDV	No. chickens in group	No.	Percentage
+	Injected	11	11	100
—	Injected	9	9	100
+	Contact	10	10	100
+	None	9	0	0

[a] 250 μg added daily to each ml of drinking water.

[b] At 60 days post-virus exposure.

Discussion. Chick embryos and chick embryo fibroblasts are employed as hosts for the growth of viruses and rickettsias used in preparation of influenza, measles, mumps, smallpox, typhus and yellow fever vaccines (1). While there is no evidence to lead one to believe that MDV is transmitted through the embryo (2, 3) there is evidence that some MDV strains might occur sequestered in chick embryo fibroblasts (4, 5) while other strains are cytolytic for this cultured cell (6, 7). In any event it is of importance to gather and to assess information concerned with the biology of viruses endogenous to hosts that serve as vaccine substrate sources, not only for its value in this area, but also for its worth in adding to information on the behavior of MDV under varying environmental circumstances.

The findings reported here demonstrate quite strikingly that penicillin as a diet supplement in drinking water conditions chickens for early MD death following MDV exposure either by injection or by contact. Streptomycin appeared to have a less striking MD enhancing effect following artificial MDV exposure (by injection) but none following a more natural MDV exposure (by contact). Thus chickens that received penicillin as a dietary supplement died of MD 5 to 7 days earlier than chickens whose diet was not supplemented with penicillin or supplemented with any of 5 other antibiotics, including streptomycin. It is important to note that the penicillin-supplemented diet employed in this study in the absence of MDV exposure was not associated in chickens with early death.

The mechanism of the action of penicillin in enhancing the course of MD in chickens is not known, but might be related to the

morphologic changes that are reported to occur in penicillin-fed chickens. Gordon, Wagner and Wostmann (8) have demonstrated in chickens on a penicillin-supplemented diet a reduced weight of the small intestine, reduced weight and lymphocyte concentration of the ileocecal tonsils and an increase in lymphocyte concentration in the thymus. These morphologic changes were not found in chickens on diets supplemented with other antibiotics (8). While these changes may explain in part our findings they are probably not significant factors in the interesting observations of Chinn (9) on enhancement of tumor development in Rous sarcoma virus-infected chickens on diets supplemented with low concentrations of bacitracin, Terramycin and Aureomycin. It is of interest to note that low concentrations of the latter antibiotic in rats was shown by Sokoloff and Eddy (10) to enhance the growth of implanted carcinomas and that unspecified levels of penicillin and streptomycin in mice were reported by Rubin and Kirschbaum (11) to increase tumor "takes" and tumor size of transplanted lymphosarcomas, but tetracycline usually failed to increase either the incidence of takes or extent of tumor growth (11).

It remains to be determined whether or not a paradoxical lead to an explanation for our findings in chickens is to be found in the report by Lewis (12) who presented evidence obtained in cell culture systems to show that the addition of penicillin to the nutrient fluid at a level sufficient to permit normal cells to grow undamaged, produced an unspecified damage to sarcomatous cells and when added at a higher level to produce an unspecified damage to normal cells, it resulted in the death of sarcomatous cells. It appears, however, that neither our observations in chickens nor those of others in rats and mice is dependent upon the well-known acute lethal effects of penicillin (13, 15) and streptomycin (14, 16) in mice and guinea pigs which are directly attributable to a marked change in the gut flora in these animals.

The broad definition in the present study of the acceleration of the clinical course of MD in chickens maintained on penicillin- and streptomycin-supplemented diets should provide worthwhile guidelines for additional work in the MDV–chicken system to define further the antibiotic-enhanced disease relationship and for investigations of other oncogenic viruses in other animal systems including careful clinical observations in man.

Summary. Use of penicillin- and streptomycin-supplemented diets was associated with an enhanced clinical course of Marek's disease in chickens after exposure to Marek's disease virus in comparison to the course of the disease in chickens on diets supplemented with Kantrex, Garamycin, Achromycin or Totacillin or on an antibiotic-free diet. The enhancement was more pronounced in chickens fed penicillin than in chickens fed streptomycin. With the former antibiotic the effect was evident following Marek's disease virus infection induced either by injection or by contact; with the latter antibiotic the effect was observed following virus injection. The significance of the findings is discussed in relation to similar enhancing effects associated with antibiotic usage in chickens infected with Rous sarcoma virus and in mice and rats with implanted lymphosarcomas.

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