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High Body Temperature of Pigeons and Sparrows As a Factor in Their Resistance to an Agent of the Psittacosis Group

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ABSTRACT

It had been previously observed that large doses of a highly virulent, avian isolate of the psittacosis-LGV-trachoma (PLT) group of organisms originally recovered from turkeys failed to affect pigeons and sparrows while readily producing lethal infections in a variety of other birds and mammals (Page, 1965). On the suspicion that the unusually high normal body temperature (43 ± 0.5°C) of pigeons and sparrows might be a factor in the resistance of these birds to this PLT isolate, the *in vitro* rates of inactivation at 43°C of the organisms infectivity for chicken embryos and the toxicity for mice were determined in comparison with an isolate of a strain normally found in pigeons and which causes disease in pigeons and sparrows. Growth rates of both isolates at 43°C in cultures of mebryonic chicken tissues were also compared.

The turkey isolate was inactivated *in vitro* at a consistently rapid rate at 43°C while the pigeon isolate was relatively resistant to 43°C inactivation. Both isolates failed to multiply in primary, embryonic chicken fibroblasts at this temperature, therefore titration of the infectivity of tissue culture homogenates at intervals after inoculation resulted only in obtaining more thermostability data. In another test, the "toxin" or mouse lethality factor possessed by the turkey isolate was slowly inactivated at 43°C whereas the "toxin" of the pigeon isolate appeared to increase for the period of the test. Lastly, a suspension of the pigeon isolate which had been heated for 24 hours at 43°C remained infectious for both pigeons and sparrows whereas the turkey isolate failed to affect either species whether or not the isolate was heated. These results suggested that because of its 43°C sensitivity, the turkey isolate was less able to survive and cause disease in birds whose normal temperature was 43°C than was the pigeon isolate.

INTRODUCTION

The wide range of hosts in which avian strains of psittacosis-LGV-trachoma* group organisms can multiply and cause disease is well known. Toxigenic strains isolated from turkeys, for example, commonly produce lethal infections when inoculated by any of several routes in small numbers into laboratory animals such as mice, guinea pigs, parakeets or turkeys. It was observed by Meyer and Eddie in 1956 that pigeons were unaffected by these virulent strains isolated from turkeys. Recently the author found that large numbers of the highly virulent NJ-1 isolate of turkey

*The psittacosis-LGV-trachoma (PLT) group of organisms currently is being studied by a group of American microbiologists with the purpose of seeking agreement among PLT research workers on the simplification and unification of PLT nomenclature. Until agreement on unified nomenclature is reached, the author will use the term PLT group of organisms.

origin failed to cause lesions in pigeons or sparrows when inoculated by the intracerebral or intraperitoneal route (Page, 1965, in press). In contract, strains isolated from pigeons or sparrows which were of low general virulence for laboratory animals produced severe systemic infection in these two avian species. While the virulence of the turkey strain could probably be attributed to its ability to produce more toxin than other strains, an explanation for the unusual species insusceptibility was sought in the possibility that the turkey strain was more sensitive to temperature approximating the normal body temperature of sparrows and pigeons (43°C) than were strains naturally infecting these birds. This paper reports results of tests designed to determine the rates of inactivation of the NJ-1 strain at 43°C in comparison with a PLT agent orginally isolated from a naturally infected pigeon.

MATERIALS AND METHODS

Isolates. The NJ-1 turkey strain was recovered by the author in 1954 from a diseased turkey (Page, 1959). The CP-3 pigeon isolate was recovered by the author in 1958 from lesionless, feral pigeons in California (Bankowski and Page, 1959).

In vitro experiments. In two tests, each isolate were propagated in the yolk sac of chicken embryos, and the infected yolk sacs harvested, homogenized and suspended in broth. Decimal dilutions through 10^{-8} of each suspension were prepared and 1ml of each dilution was inoculated into the yolk sac of 6-day-old chicken embryos, 6 eggs per dilution. The orginal undiluted suspensions were then divided in half and incubated, one at 43°C and one at 37°C. In the first test, aliquots of each suspension were removed at intervals of 2, 4, 6 and 24 hours after incubation, decimally diluted and inoculated in embryonated chicken eggs. In the second test, aliquots were removed at intervals of 3, 6, 9, 12, and 24 hours after the start of incubation, diluted and inoculated into eggs. In the third *in vitro* test, freshly harvested suspensions of each isolate were incubated at 43°C with control at 37°C, and at zero, 5 and 12 hours aliquots of each suspension were removed, decimally diluted and inoculated intravenouly into 16 gram Swiss-Webster (5 per dilution) as well as into embryonated chicken eggs. Incubated eggs were candled daily and embryonic deaths recorded. After 14 days incubation at 37°C, all survivors were killed and discarded.

Inoculated mice were checked four times daily and those dying within 48 hours from the time they were inoculated were considered to have died from the effects of "toxin" possessed by PLT isolates. Mouse lethal 50 percent endpoints were calculated upon the basis of these deaths. Those dying after the 48th hour were considered to have succumbed to the several effects of multiplication of the organisms, rather than toxicity of a given number of organisms (Meyer and Eddie, 1956).

Lethal 50 percent endpoints were determined for each titration according to the method of Reed and Muench (1938).

In vivo experiments. Attempts were made to compare the growth rates of both isolates in primary cultures of embryonic chicken fibroblasts incubated at 43°C (with controls at what microscopically visible effects, if any, high temperature incubation had on normal cells. No effect was observed for four days except that the fibroblasts appeared to multiply faster for the first two days at 43°C than at 37°C. By the fifth day at 43°C signs of degeneration of cells began to appear. In the test proper, 72 four-ounce perscription bottles containing fibroblast sheaths were infected with either the turkey or pigeon PLT isolate, 36 bottles per isolate. Eighteen bottles infected with each isolate incubated at 43°C and 18 at 37°C. At zero, 6, 24, 30, 48, and 72 hours after inoculation the cells and supernatant fluids from three bottles of each group were combined, homogenized and decimally diluted. Aliquots from each dilution were inoculated in embryonated chicken eggs, six per dilution. The inoculated eggs were candled daily and embryonic deaths recorded for LD_{50} determinations.

Bird inoculations. Samples of heated (43°C, 24 hrs.) and unheated suspensions of both strains were inoculated in 0.5 ml volumes intraperitoneally into adult pigeons and sparrows, 4 pigeons and 5 sparrows per suspension.

RESULTS

In three separate experiments, suspensions of both the turkey and pigeon isolates were incubated at 43°C (with controls at 37°C and aliquots were removed at various intervals, decimally diluted and inoculated into chicken embryos to determine the number of embryo lethal 50 percent units remaining in each ml of the original suspensions. These results are tabulated in Table 1. Results of experiment two, as a typical example, are expressed graphically in Figure 1.

In all experiments, the turkey isolate was inactivated faster at 43°C than was the pigeon isolate. In experiment one, the titer of the pigeon isolate suspension held at 43°C remained relatively constant after a slight early decline whereas the titer of the turkey isolate suspension steadily decreased. In the second experiment, the turkey isolate was completely inactivated within 24 hours while the pigeon isolate suspension had a high titer ($10^{5.7}$) after that period. In the third experiment, embryo lethal titers and mouse lethal titers of the suspension of the turkey isolate declined during 12 hours incubation at 43°C while the pigeon isolate titers remained constant or actually increased.

Titers of control suspensions held at 37°C decreased at a much slower rate. The rates at this temperature were similar for both isolates except that a slight rise in titer occured in some suspensions of the pigeon isolate after 4-6 hours incubation.

The results of titrations of infected tissue cultures in embryonated eggs are found in Table 2. The numbers of embryo lethal units remaining in the cultures incubated at 43°C decreased slowly for 72 hours (except for a slight rise at the 6th hour after incubation), indicating that both strains failed to multiply at this temperature. At 37°C

Table 1. *Titers of egg-propagated suspensions of turkey and pigeon isolates incubated for various intervals at* 43 or 37°C.

		Log $_{10}$ embryo LD_{50}/ml of suspension				Log $_{10}$ mouse I. V. LD_{50}/ml			
		43°C		37°C		43°		37°	
Exp.	Hrs.	Turkey Isolate	Pigeon Isolate	Turkey Isolate	Pigeon Isolate	Turkey Isolate	Pigeon Isolate	Turkey Isolate	Pigeon Isolate
1	0	6.00	6.38	6.00	6.38				
	2	6.00	6.16	—	—				
	4	5.68	6.16	5.68	6.57				
	6	5.38	6.16	—	—				
	24	—	—	4.63	5.17				
2	0	7.68	7.00	7.68	7.00				
	3	7.17	6.59	—	—				
	6	6.47	6.52	7.00	7.17				
	9	6.22	—	—	—				
	12	5.20	6.38	6.84	7.18				
	24	0	5.17	5.50	6.30				
	48	0	0	3.30*	4.40*				
3	0	8.68	9.38	8.68	9.38	2.50	3.00	2.50	3.00
	5	8.38	8.83	8.38	9.17	2.83	3.15	2.83	3.25
	12	7.63	9.38	8.17	8.63	2.17	3.25	2.63	3.30
	Log Change (0-12hrs)	—1.05	—0.00	—0.51	—0.75	—0.33	+0.25	+0.13	+0.30

*Endpoint was not reached, but titer was estimated on basis of average-day-to-death data.

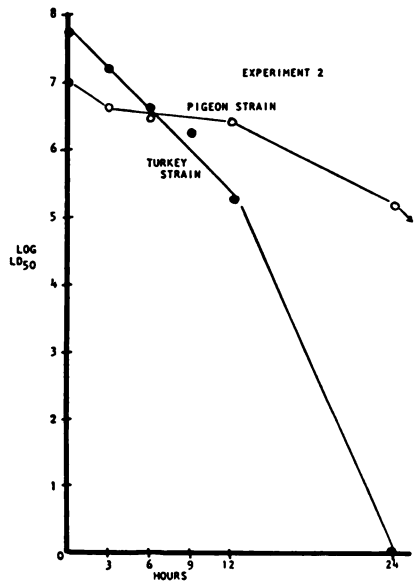

Figure 1. *Inactivation curves of the infectivity of suspensions of the turkey and pigeon PLT isolates at 43°C.*

however, titers of infected tissue cultures rose 30 hours after inoculation thereby demonstrating a multiplication cycle that is typical of avian strains of PLT organisms.

Inoculation of unheated suspensions and 24-hour-43°-heated suspension of the pigeon isolate into five sparrows and four pigeons per suspension resulted in the developmnt within two weeks of severe lesions typical of PLT infection. Swollen liver and spleen, thickened airsacs, with viscera and airsacs covered with fibrinous exudates containing numerous monocytic inflammatory cells were frequently observed. Typical elementary bodies of the agent were observed microscopically in the cytoplasms of numerous monocytes. The agent was reisolated by subinoculation of homogenized tissues from affected birds into chicken embryos. Serums from blood collected at termination were tested for complement-fixing PLT antibodies. Positive serums were found in 3 of 8 pigeons and 4 of 10 sparrows. Sparrows and pigeons similarly inoculated with the turkey strain failed to develop detectable PLT antibodies or gross lesions typical of PLT infection and the agent was not reisolated from any of the birds.

DISCUSSION

Since the infectivity and toxicity of the turkey isolate was more heat labile than that of the pigeon isolate, it was conceivable that 43°C sensitivity could be a factor contributing to the turkey isolate's failure to cause disease in pigeons and sparrows. Although both isolates were ultimately inactivated at that temperature, the rate of inactivation was faster for the turkey isolate. This heat lability might be important in the first hours after infection when survival of the invading organism is at the greatest risk.

Table 2. *Titers of suspensions of tissue-culture-propagated organisms grown at 43 or 37°C.*

	Log_{10} embryo LD_{50} per ml of suspension			
	43°C		37°C	
	Turkey isolate	Pigeon isolate	Turkey isolate	Pigeon isolate
0	4.63	5.00	4.63	5.00
6	5.38	6.00	5.32	5.68
24	3.83	5.32	3.63	4.68
30	3.63	3.80	6.22	5.00
48	3.17	3.37	6.50	6.50
72	2.63	2.17	5.63	5.83

Previous experiments offer a correlative sidelight. When adult turkeys whose normal body temperatures vary between 39-41°C (depending upon the ambient temperature) were experimentally infected with the same turkey isolate as used above, they developed elevated temperatures ranging from 41-43°C. Those birds that survived the infection (usually after a two week febrile period) were found to have eliminated the organisms from their tissues instead of developing a chronic state typical of psittacosis in other birds (Page, 1959). In these cases, cellular and newly acquired immunity probably were more significant factors, but body temperatures approaching 43°C may have assisted the process of reducing the number of infecting organisms.

In the case of pigeons and sparrows, genetically controlled resistance factors probably play a larger role in the resistance of these avain species than does the simple matter of temperature protection. Nevertheless, one might conclude that the pigeon isolate is better able to survive in birds with unusually high normal body temperatures than is the turkey isolate.

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CONFERENCES NOTICES

TITLE	*DATE AND PLACE*	*FOR INFORMATION WRITE*
46th annual *Conference of Research Workers in Animal Diseases.* (Attendance is limited to elected members and certain invited guests.)	Nov. 29-30, 1965 Midland Hotel, Chicago, Illinois	Dr. A. F. Weber Coll. Vet. Med. Univ. of Minn. St. Paul, Minn.
8th International Congress of Comparative Pathology (ICCP) (Deadline for submission of papers is around December 31, 1965)	Sept. 11-18, 1965 Beirut, Lebanon	Dr. J. K. Frenkel Dept. of Path. Univ. of Kansas Medical Center Kansas City, Kans. 66103