Escherichia coli in Normal and Traumatized Tissues.*† (31219)

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While normal tissue is usually assumed to be sterile in the absence of manifest disease, McCarthy *et al*(1) indicated that small numbers of bacteria are present in normal tissue of 40-50% of the birds examined and that 61-74% of poultry bruises harbored relatively large numbers of both aerobic and anaerobic bacteria. Analysis of the bacterial flora of bruised tissue revealed the presence of pathogenic and nonpathogenic organisms in these tissues and that 22% of the predominant isolates were Gram-negative rods which included coliforms.

Hamdy *et al*(2) established that the gut, air sac, and skin of birds(1) were both sources of these bacteria and possible entry sites to the traumatized areas. These damaged tissues were ideal environments for organisms following bruising and in the early stages of healing. Hamdy and Barton(3) determined the fate of a pathogenic marker strain (MS) of *Staphylococcus aureus* injected intramuscularly (IM) into both control and traumatized tissue. These authors showed that the control tissue possessed a highly active clearing mechanism against this pathogenic organism, whereas bruised tissue stimulated and supported its growth. Time-course studies also showed that *S. aureus* (MS) persisted, in large numbers, in bruised tissue for 18 days in the absence of noticeable infection. The experiments in this communication were designed to examine factors affecting the persistence of a nonpathogenic *E. coli* culture injected IM into normal and traumatized poultry muscle.

Materials and methods. Experimental birds and bruising procedure. Apparently normal White Leghorn chickens, 8 to 10 weeks old, weighing approximately 3-4 lb were maintained in batteries at a constant temperature of 22°C on standard rations free of medication with water *ad libitum*. The feathers over the breast muscle were plucked and, unless otherwise stated, the pectoralis major muscle was contused using the standard three-blow technique described by Hamdy *et al*(4). Symmetrically located areas on different birds were used as controls (unbruised). Severe bruises were produced using 5 blows; medium bruises, 3 blows; and superficial, 1 blow.

Bacterial culture. A nonpathogenic culture of *E. coli* K-12 (obtained from W. J. Payne, Bacteriology Dept., Univ. of Georgia) was maintained by repeated transfer in nutrient broth (Difco) in test tubes. Flasks of nutrient broth were inoculated with 1 ml of an active culture and incubated for 18-24 hours at 37°C. The cells were harvested by centrifugation, washed 3 times, and resuspended in sterile physiological saline at the desired concentration.

Injection and sampling technique. The injection of *E. coli* and sampling techniques were performed as previously described for *S. aureus*(3). The number of viable organisms was determined by plating the appropriate dilutions on violet red bile agar. The site of injection was marked by applying blue dye to the outside of the needle before injection.

Preparation of extrastromal hemoglobin. This solution was prepared as described by Shinowara(5).

Disc-sensitivity procedure. Nutrient agar (Difco) was seeded with 1% inoculum of an 18-hr active culture of the test organism. Small volumes of solution (0.05 ml) containing the desired concentration of extrastromal hemoglobin were added to sterile discs placed on the surface of the agar. The plates were incubated at 37°C, and examined after 48 hours for stimulation or inhibition of growth. Diameters of the areas surrounding the disc, which contained a greater concentration of growth of the culture (stimulation) or no growth (inhibition) as compared to control areas were measured in mm.

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TABLE I. Comparative Survival of *E. coli* K-12 in Two-Day-Old Bruised and Control Tissues.*

Bird No.	No. of viable cells/g tissue Control	Bruise
1	0	0
2	0	0
3	0	5
4	0	20
5	0	17.5×10^6
6	0	51×10^6
7	10	111×10^6
8	10	130×10^6
9	20	157×10^6
10	350	170×10^6
11	—	216×10^6
12	—	320×10^6
Avg	39	98×10^6

* Birds were injected intramuscularly with an *E. coli* suspension containing 48×10^4 cells.

Results. Survival of E. coli *in traumatized tissue.* Twelve traumatized and 10 normal birds were injected with an *E. coli* suspension containing 48×10^4 viable cells. Two days after injection, all the chickens were sacrificed and the tissues surrounding the site of injection were excised and *E. coli* enumerated. The results (Table I) showed that injected tissues of normal birds showed a decrease in organisms as indicated by the low average count of 39 cells/g tissue within 2 days post-injection. It should be pointed out that 60% of the tissues examined had no viable bacteria. Thirty percent contained less than 30 cells/g tissue and 10% had 350 cells/g tissue. The survival of *E. coli* in traumatized tissue represented a different pattern. These tissues supported the growth of the organism, as evidenced by an average of 98×10^6 cells/g tissue. It is of interest to note that the traumatized tissues of only 2 birds failed to support the organisms and another 16.6% had less than 30 cells/g tissue. However, 66.8% contained more than 100-fold the original number of *E. coli* injected.

Fate of E. coli *in normal and traumatized tissue as a function of time post-injection.* Ninety-eight traumatized and 47 control birds were injected IM with 48×10^4 cells of *E. coli.* Birds were killed at intervals and injected tissues examined for number of organisms. The results (Fig. 1) showed that the population of *E. coli* in normal tissues decreased rapidly within one day from 48×10^4 to 22 cells/g tissue. The loss of organisms from normal tissues continued at a much slower rate on the second, third, and fourth days. No viable cells could be recovered from the tissues on the fifth day, again indicating that the tissue of normal birds seems to possess a clearing mechanism that is highly active against *E. coli* K-12. The highest cell count in normal tissue (350 cells/g tissue) was observed on the second day. In the traumatized area the number of *E. coli* organisms increased rapidly on the first and second days and maintained this level until the seventh day followed by a slight decrease thereafter to the ninth day. The highest count recovered (72×10^7 cells/g tissue) was noted on the fifth day.

Analysis of the number of organisms recovered from normal and bruised tissues (Ta-

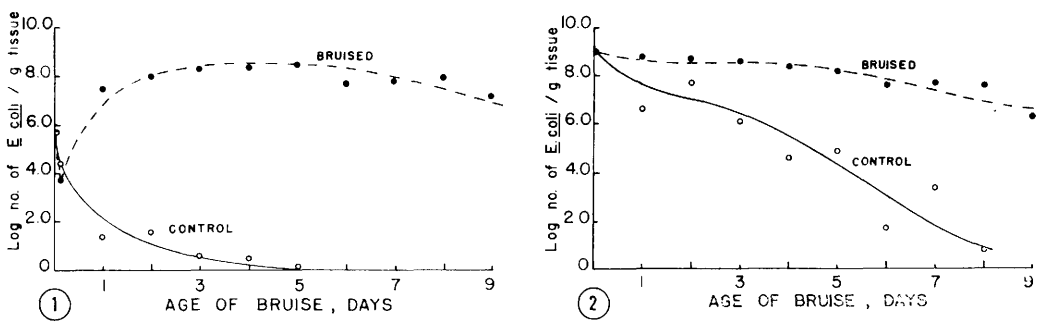

FIG. 1. *E. coli* in control and bruised tissues as a function of time post-injection. Results are expressed on a daily basis as the mathematical averages of the *E. coli* counts in bruised and control samples.

FIG. 2. Effect of a large number of cells in the inoculum on the behavior of *E. coli* in control and bruised tissues as a function of time post-injection. Results are expressed on a daily basis as the mathematical averages of the *E. coli* counts in bruised and control samples.

TABLE II. Distribution Analysis of Tissue Samples Containing Specified Log Numbers of *E. coli* Cells Recovered from Normal and Traumatized Poultry Tissue at Various Time Intervals Following Injection with 48×10^4 Cells.

Days post inj	Normal				Traumatized									
	No. of birds	Log No. of cells/g tissue 0–1	1–2	2–3	No. of birds	Log No. of cells/g tissue 0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9
1	10	3	7		11			3			1	1	6	
2	10	6	3	1	12	3	1						2	6
3	10	8	2		11	1	1						2	7
4	10	8	2		12	1	2						3	6
5	7	7			12	2								10
6					12	5						1	3	3
7					12	3	2	1		1			3	2
8					12	6							3	3
9					4	1							1	2
Totals:	47	32	14	1	98	22	6	4		1	1	2	23	39

ble II) indicated that 97.9% of the control (normal) birds had a count of less than 100 cells/g tissue. The bruised tissue birds presented two distribution groups. One group of 28.6% of the bruised birds had a count of less than 100 cells/g tissue; the other, representing 63.3%, had more than 1×10^7 cells/g tissue, *i.e.*, more than the number in the injected dose.

Persistence of E. coli *in traumatized tissue.* The fate of injected organisms upon complete healing of the bruised tissue was investigated by extending the microbial analysis of the tissue to 18 days. Seventy-two bruised birds were injected at the center of the bruise with a saline suspension of *E. coli,* each bird receiving 625×10^5 cells. These birds were sacrificed and their tissues assayed for the number of organisms daily beginning on the tenth day and continuing for 18 days. The results indicate that the organisms were able to persist in the traumatized area throughout the experiment. It should be pointed out, however, that the number of organisms recovered from these tissues declined with time. In fact, 79% of all the birds examined had a viable count of less than 600 cells/g tissue. Of these, apparently 56% succeeded in eliminating the test organism completely from their tissues. It was also found that 15 birds out of the 72 samples examined showed a count of more than 100 cells/g tissue.

Studies were then undertaken to examine some of the factors that may influence the survival and fate of *E. coli* organisms in tissues of normal and experimentally bruised birds.

Effect of number of cells in the inoculum. Increasing the number of *E. coli* cells injected to 12×10^8 did not significantly increase the number of *E. coli* cells recovered from bruised tissues (Fig. 2). However, 14% more of the bruised tissues examined had counts above 1×10^7 when the larger inoculum was used and 17% less of the samples had counts below 10 cells/g tissue (Table III). Normal tissue again eliminated the culture but at a slower and steadier rate. On the eighth day *E. coli* was still present in some samples but only in small numbers.

Effect of hemoglobin. Previous experiments showed that traumatized tissue enhanced the growth of *E. coli* for 8-9 days following injection but that control tissues supported growth poorly even when a high number of cells was injected. The large amount of blood in bruised tissues led to an attempt to determine the role of hemoglobin on the activities of the test culture using the disc-sensitivity procedure. The results showed that extrastromal hemoglobin stimulated the growth of *E. coli,* but that this stimulation was linearly dependent on the hemoglobin concentration only at levels ranging between values of 4×10^{-3} to 16×10^{-3} μM/disc.

Discussion. Knowledge of persistence of pathogenic or nonpathogenic bacteria in tissue is incomplete. More information concerning organisms associated with poultry bruises is important in better understanding

TABLE III. Analysis of Tissue Samples of *E. coli* Cells from Normal and Traumatized Poultry Tissues at Time Intervals Following Injection with 12 × 10^8 Cells.

Days post inj	Normal										Traumatized									
	No. of birds	Log No. of cells/g tissue									No. of birds	Log No. of cells/g tissue								
		0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9		0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10
1	9				3	1	1	3	1		10	1		1						5	3
2	10			2	1		1	2	3	1	9	1						1	2	3	2
3	10			3		1	4	2			10	1								8	1
4	9		2	4	1		2				9								2	7	
5	9	1	3	2	1		2				9								5	4	
6	9	7	1	1							9						1	1	7		
7	8	5	1		1	1					8	1		1				1	3	2	
8	3	2	1								9				2	1	2		2	2	
9											2						1	1			
Totals:	67	15	8	12	7	3	10	7	4	1	75	4	0	2	2	1	4	4	21	31	6

of the role of these injured tissues as a possible source of contamination to other birds and to poultry workers and may have far-reaching biologic and epidemiologic significance. Price *et al*(6) and Smibert *et al*(7) examined natural cases of aerosaccitis in poultry and found *E. coli* to be among the predominant organisms isolated from the lung and air sacs and from pericardial tissues exhibiting lesions. The response of normal poultry tissue to IM injection of nonpathogenic *E. coli* was a decrease in the numbers of these organisms. Several possible explanations are offered to account for this response. Both humoral and cellular defense mechanisms in these birds may be highly active against this culture. It is also possible that the tissue microenvironment is lacking in essential nutrients required for their growth or that these tissues contain agent(s) or unfavorable conditions that react with *E. coli* cells rendering them easily destroyed or limiting their reproduction. The latter speculation is supported by Hirsch(8) who established the presence of many antibacterial substances such as lysozyme, β-lysine and lactic acid in healthy tissues. Skarnes and Watson(9) also stated that antibacterial basic proteins and polypeptides in normal tissue may combine with the cell nucleoprotein of the bacteria through salt bonding, thus disrupting some of the important cell functions. Traumatized tissue, on the other hand, supported the growth of the test culture. Time-course studies of *E. coli* in tissue post-trauma showed that *E. coli* counts at any given time, particularly in tissues injected with 48 × 10^4 cells, fell in 2 distinct groups: one represented 30% of the birds examined and had a count of less than 100 cells/g tissue, the other had a count of 1 × 10^7 cells/g tissue. This may be due to differences in susceptibility to the test culture as pointed out by Gross(10) who reported that birds infected with both PPLO (pleuropneumonia-like organisms) and NDV (Newcastle disease virus) were more susceptible to *E. coli* invasion than non-infected birds.

The enhancement of *E. coli* growth in the bruised tissue may also be due to the composition of the environment, particularly the presence of extrastromal hemoglobin which

was found to stimulate the growth of this culture *in vitro*. Again it should be pointed out that an equilibrium between growth and destruction rates of the test organisms was evident in the tissue even when large numbers of cells were injected. An attempt was made to study the effect of severity of the trauma on rate of growth of the test culture. However, the results revealed that differences in growth pattern of *E. coli* in the traumatized tissue as related to force applied to inflict the bruises (severe, medium or superficial) were slight.

The studies reported here demonstrate the persistence of *E. coli* in small numbers in traumatized tissue for 18 days, but the epidemiological significance of this observation is yet to be determined. It is also possible that the survival of bacteria in tissue could give rise to clinical disease as pointed out by Payne and Derbyshire(11).

Summary. A known number of *E. coli* K-12 cells was injected in bruised and normal poultry tissues and examined daily thereafter. Normal tissues completely eliminated 48×10^4 *E. coli* cells within 5 days but were unable to eliminate 12×10^8 cells within 8 days. However, the number of cells recovered decreased with time and very few cells were recovered on the 8th day. Regardless of the number of cells injected, the majority of bruised tissue stimulated and supported *E. coli* growth for one week. At the end of 18 days post-injection, a small percentage of bruised tissues still harbored the test culture. Although extrastromal hemoglobin stimulated *E. coli* growth, increasing the severity of the bruise (and therefore the amount of blood in this tissue) did not affect *E. coli* growth significantly.

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Effect of Triton* Ingestion on Fat Retention, Blood Lipids and Growth in Rats.† (31220)

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In an earlier study it was reported that absorption of fat was delayed when fat was fed with orally administered Triton(1). Serum lipid levels were found not to be elevated when a fat-Triton mixture was fed(2), and the lower serum lipid levels were explained by a decreased fat absorption as a result of a marked inhibition of lipolysis by pancreatic lipase in the presence of Triton.

If the Triton itself is not absorbed and it decreases the absorption of dietary fat, the intake of Triton along with a meal might serve as a means of reducing one's caloric intake by lowering fat absorption. Following fat retention during a definite period would provide important data as to whether and to

* Triton WR-1339 (oxyethylated tertiary octyl phenol formaldehyde polymer), Winthrop Laboratories, is a detergent which has been used as an aid in studying fat metabolism.

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