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Longevity of Bact. Tularense in Muscle Tissue.

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As the result of investigations carried out by Francis, it has become customary to test for the presence of *Bact. tularense* in animals dead from tularemia by the injection of a suspension of liver or spleen into guinea pigs. Francis[1] has pointed out that *Bact. tularense* gradually disappears from the liver and spleen of animals dead from tularemia. He[2] has demonstrated that *Bact. tularense* in spleen tissue may be kept viable 30 days or longer by storage in 50% glycerine at ice-box temperature. He states that liver is inimical to the life of the infection and, when stored in glycerine with spleen, will destroy the infectivity of the spleen tissue. In his investigation of rabbit livers from the Washington Market, Francis[3] found *Bact. tularense* viable in rabbits shipped from Tennessee, but the lapse of time after death was unknown.

We have investigated the longevity of *Bact. tularense* in the liver and spleen of animals dead from experimental tularemia and have gathered comparative data with respect to the longevity of the organism in muscle tissue. Tissues from one guinea pig and 2 rabbits were injected at intervals into 142 guinea pigs used as test animals.

Test No. 1. The carcass of a guinea pig dead from experimental tularemia 4 days after inoculation was kept at room temperature (ca. 20°C.). The spleen and liver were thickly studded with necrotic areas. At intervals portions of the liver and spleen of approximately the same size were removed and emulsified in normal saline solution. The suspensions were immediately injected subcutaneously into guinea pigs. The results are shown in Table I.

Test No. 2. The carcass of a rabbit dead from experimental tularemia was used. The rabbit died 4 days after inoculation, with both inguinal glands enlarged and the liver and spleen well covered with necrotic areas. The carcass was stored at 6°C. with the organs *in situ.* At intervals given below, small pieces of liver, spleen and muscle were removed and weighed. Two suspensions were made, containing 0.1 gm. tissue per cc. and 0.01 gm. tissue per cc., respec-

[1] Francis, Edward, Bulletin No. 130, Hygienic Laboratory, 1922, 6.

[2] *Ibid., J. Am. Med. Assn.*, 1928, xci, 1155.

[3] *Ibid., J. Am. Med. Assn.*, 1925, lxxxiv, 1243.

TABLE I.

Duration of storage	Guinea pigs inoculated	Liver	Spleen
Fresh	2	+	Not done
2 days	2	+	Not done
3 "	1	+	+
4 "	1	—	—
5 "	1	—	+
6 "	1	—	—
7 "	1	—	Uns.
9 "	1	—	Uns.

+ Guinea pig died with lesions typical of tularemia.
— Guinea pig chloroformed after at least 3 weeks; no evidence of tularemia.
Uns.—"Unsatisfactory." Guinea pig died of septicemia within 36 hours.

TABLE II.

Duration of storage in icebox	Liver		Spleen		Muscle	
	0.1 gm	0.01 gm.	0.1 gm	0.01 gm.	0.1 gm	0.01 gm.
Fresh	+	+	+	+	+	+
3 days	+	+	+	+	+	+
5 "	+	+	+	+	+	+
7 "	+	+	+	+	+	+
8 "	—	—	+	+	Not done	
10 "	—	—	—	—	+	+
12 "	—	—	—	—	+	+
24 "					+	+
31 "					—	—
33 "					+	—
36 "					—	—

tively. Much difficulty was experienced in suspending the muscle tissue in normal saline solution, and this was imperfectly accomplished. The suspensions were injected immediately after preparation. The results obtained are found in Table II.

Test No. 3. A rabbit carcass was stored at 6°C. under the same conditions as the carcass in Test No. 2. This rabbit died 4 days after inoculation, with left inguinal gland enlarged and the liver and spleen thickly studded with necrotic areas. Liver, spleen and muscle tissue suspensions were injected into guinea pigs in amounts of 0.1 and 0.01 gm., prepared as above. The results of this experiment are shown in Table III.

While these results are obtained from 3 carcasses only, they give some indication as to the persistence of the infection in the tissue of a dead rabbit or guinea pig. They show a remarkable persistence of the viable organism in muscle as compared with liver and spleen. Consistent results observed for muscle tissue during the first 24 days, indicate that the variable results observed for liver and spleen are dependent upon actual changes taking place in the tissue. Inasmuch as negative results were obtained as early from the spleen as

TABLE III.

Duration of storage in icebox	Liver		Spleen		Muscle	
	0.1 gm.	0.01 gm.	0.1 gm.	0.01 gm.	0.1 gm.	0.01 gm.
Fresh	+	+	+	+	+	+
2 days	+	+	+	+	+	+
3 "	+	+	+	+	+	+
4 "	+	+	+	+	+	+
5 "	+	+	Uns.	+	+	+
6 "	+	+	+	—	+	+
8 "	—	—	—	—	+	+
10 "	+	+	None left		+	+
12 "	—	—			+	+
14 "	Uns.	+			+	+
17 "	—	—			+	+
20 "	—	—			+	+
22 "					+	+
24 "					+	+
29 "					+	+
33 "					+	—
35 "					+	—
38 "					—	—
42 "					—	—
46 "					—	—

from the liver, no marked difference in the viability of *Bact. tularense* in liver and spleen tissue of rabbits is discernible from our data. These tissues may not be infective on the eighth day after death when stored at 6°C. It appears that rabbit muscle, under the same conditions, will retain its infectivity consistently for about 4 weeks and may be infective for as long as 35 days.

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Effect of Changes in Environment on Development of the Chick.

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The work of Stockard in varying the normal environment of developing eggs with resulting malformations suggested carrying out similar experiments on a warm blooded animal, such as the chick. The eggs were placed in an incubator for 16 to 18 hours prior to operation. A piece of shell was removed, and in some instances 3 drops of 1/1000 solution of 95% alcohol were added to the side of the embryo, in others, 3 drops of normal saline (both at room and incubator temperatures), while a few eggs were carried along as controls.