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Studies on anthrax infection.

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The unsettled problems of infection with *B. anthracis* are very many. Besredka has recently declared his belief that the cutaneous is the only route of infection. Intestinal anthrax is the commonest form in which the natural disease appears to be found. The question has been raised whether there is such an infection. The opposing view is that the findings and symptoms in the intestines are only part of a general infection and that the portal of entry is in the skin, usually about the mouth and nose, and the local reaction, if any, is overlooked. In carrying out some experiments to cover some of the doubtful points we undertook the feeding of guinea pigs, taking particular care not to contaminate the mouth cavity. The technic used by previous workers did not seem satisfactory so a simple method was tried which protected against these possible contaminations. Small gelatine capsules were filled with the culture to be used, the mouth was opened and the capsules placed in the back of the throat. The animal swallowed them promptly. A guinea pig was fed in this way with a virulent culture rich in spores and a daily culture of the feces was made in agar after heating to 75°C for 15 minutes. A fecal pellet was emulsified in 10 c.c. of saline and 1 c.c. was used for the plate. The colony characteristic of *B. anthracis* is easily recognized but isolations were made each day and tested on fresh animals. In this way we found virulent anthrax spores in the feces up to the seventh day. Two weeks after the feeding the animal was given a subcutaneous dose and died of anthrax. A second pig was fed in the same way and positive fecal cultures were obtained up to the fifth day. An accident resulted in this animal's death on the eighth day but no microscopic or cultural evidence of anthrax could be found. Four guinea pigs fed in the same way were studied up to the twenty-ninth day when they were killed by a subcutaneous route injection. These animals showed anthrax spores up to the tenth day

with negative intervals between. A number of other fed guinea pigs are still under observation. The results of these tests would indicate that the intestinal tract is not an easy portal of entry for *B. anthracis;* that healthy susceptible animals can carry virulent anthrax bacilli for a considerable time and by the distribution of the spores in their feces can be a ready means of spread.

In reading the literature it is evident that the portal of entry is frequently in doubt. In injecting rabbits subcutaneously in the ear we noted that the local reaction was often scarcely noticeable and that the characteristic edema only appeared in the cervical tissues some distance from the portal of entry and the amount was not always very pronounced. The explanation suggested itself that the firmer tissues of the ear did not allow the edematous fluid to collect at the point where the organism was establishing itself. In the fluid are numerous leucocytes and these may also be prevented from collecting in quantity. Is it not possible therefore to inject in such tissues a dose of anthrax spores too small to be fatal in the looser tissues but which can establish themselves where some of the defenses are held in check? To determine this point we took six guinea pigs and injected them with about 50 spores in 1/20 of a cubic centimeter of saline. Three were injected subcutaneously over the abdomen and three under the skin of the ear. The three former lived and two of the latter died, one in forty-seven hours, and one in eighty-four. It is to be noted that the guinea pig dying first had accidently received an extremely small dose. The point of the needle was seen to be through the ear when the injection was started and the needle was withdrawn at once and so, although undetermined, the amount in the wound must have been minute. The reason the needle was withdrawn was to prevent any undue irritation which would have interfered with the object of the experiment. The autopsy findings in both these animals would not have suggested where the injection had been made—a slight amount of edema on the side of the neck in one and a slight amount along the trachea in the other. These are suggestive findings and will need to be repeated many times.

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The comparative fat content of the portal vein as determined by the presence of fat particles with the darkfield microscope.

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Although it is generally stated that not all of the fat ingested can be recovered from the thoracic duct, there is no very satisfactory evidence to show that fat is absorbed *directly* into the blood under normal conditions. Fat absorption through the villi into the lacteals and thoracic duct as the "molecular basis of chyle" and the entrance of these minute particles (chylomicrons) into the venous system has been known since the time of Boyle, Hewson and Gulliver, and is not difficult of demonstration.

The use of the darkfield microscope has been of distinct value in studying the increase and decrease of the chylomicrons during, and after, the period of fat absorption with the use of the ocular micrometer, following the method of Gage for counting these minute particles with approximate accuracy.

In connection with other study upon fat absorption, observations were made upon the chylomicron content of the blood of the aorta, jugular and portal veins at different periods of digestion with results as shown in the following table:

CHYLOMICRONS

Animal	Digestion	Aorta	Jugular V.	Portal V.
Kitten	6 hours	75	.....	59
Kitten	4 hours	132	.....	48
Kitten	7 hours	192	.....	126
Cat	3½ hours	142	117	45
Kitten	6 hours	35	26	1
Kitten	7 hours	121	100	76
Cat	18½ hours	90	100	22
Dog	4 hours	13	15	5
Dog	24 hours	11	.....	1.5
Cat	5½ hours	8	25.8	2
Cat	4 hours	100.2	152.2	33.4
Kitten (600 gms.)	4 hours	56.2	66.8	15