AN INVESTIGATION OF THE STERILITY OF FISH TISSUES[1]

B. E. PROCTOR AND J. T. R. NICKERSON

Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts

Received for publication, May 9, 1935

The bacteriological examination of fish tissues to determine whether or not bacteria are present has concerned numerous investigators for over three decades. Müller (1903) reported that the flesh of perch and carp was sterile. Ulrich (1906) concluded that bacteria were present in each of the 40 cod and sole studied by him. The same results were reported in respect to perch, pike, dace, salmon, tench, carp and barb. Bruns (1908), working with fresh, salted, dried and smoked fish, found no bacteria in deep tissues but occasionally found them in surface samples of flesh.

Browne (1918) concluded that normal fish muscle was sterile. Hunter (1920), from an examination of various species of Pacific salmon, stated that flesh from the belly or back of these fish was sterile if taken within two hours after the fish was removed from the water. He also found the blood of fresh salmon to be sterile.

Harrison, Perry and Smith (1926) reported that the flesh of haddock was sterile when examined within three hours after the fish were caught.

Fellers (1926) examined the flesh of Pacific salmon and was unable to obtain bacteria from the muscle of those freshly caught. He also found that bacteria were usually absent from the stomach and intestines of those salmon well advanced in their spawning migration.

Stewart (1930) made 143 cultures from codlings. Bacteria

[1] Contribution No. 60 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

were obtained in only 5 of these cultures. These organisms were found to be similar to those occurring in slime on fish and were therefore regarded as contaminants.

Gee (1927) (1930) reported the presence of bacteria in fresh fish muscle. In his earlier report he was consistently able to isolate a spore-forming organism from the flesh of both freshly caught and spoiled haddock. In a later paper he cited the presence of bacteria in the flesh of certain freshly caught haddock, cod and dogfish.

The literature cited would seem to favor the opinion that the flesh of fresh fish is sterile, although conflicting evidence is presented in respect to haddock. In view of the fact that haddock is the most important food fish taken in New England, further information seemed desirable.

The present investigation is concerned primarily with the examination of haddock (*Melanogrammus aeglifinus*). The fish used were, as soon as removed from the water, placed at such a temperature that they were solidly frozen within an hour and were kept below −17.8°C., until examined one to eight weeks later.

SECTION I

The first experiments were conducted on 3 small haddock and 3 large haddock.

Areas were first marked off on the skin of a still frozen fish with a sterile scalpel. The areas laid out on the 3 large haddock were (a) in the anterior region just back of the head, (b) the middle lateral portion, and (c) the caudal region. The same was done on both sides of the fish. A total of 90 samples was taken.

The smaller haddock were of such a size (2 pounds) that samples could be conveniently taken only from the areas in the middle region on both sides of each fish. The number of muscle samples taken from these haddock was 30.

The skin covering these areas was pulled off with pliers. A flap of the underlying surface muscle was then cut away and discarded. A sample of the sub-surface muscle weighing approximately 5 grams was then removed with a sterile metal gouge.

Various dilutions were made from these muscle samples after they had been disintegrated by shaking in sterile water.

From the same areas of the fish, samples of approximately one gram in weight were removed in an identical manner and placed in sterile Petri dishes and in sterile broth tubes. Four 1 gram samples were taken from each area and used as inocula for two plates and two tubes of 0 dilution.

Dilutions

Dilutions (from 1:10 to 1:1,000,000) were used to make duplicate agar plates and duplicate broth tubes.

Culture media

Fish broth and fish agar were used as culture media. Five hundred grams of haddock muscle were ground in a meat grinder and extracted for one hour with 1000 cc. of tap water. This mixture was boiled for five minutes, then filtered and autoclaved to precipitate some of the protein. After filtering a second time, the resulting solution was tubed and sterilized to be used as fish broth. Solid media was made by adding 1.5 per cent of agar to the broth.

Plain nutrient broth and nutrient agar were used in the experiments reported in sections II and III.

Incubation period

All cultures were incubated for one hundred and sixty-eight hours at 20°C., before observations were made to determine the presence or absence of bacterial growth.

Results

The bacteriological results obtained from the examination of 90 samples of muscle excised from 3 large haddock and 30 muscle samples from 3 small haddock may be summarized as follows: A total of 252 plates of various dilutions were made from the muscle of the three large haddock; 246 of these plates were sterile. Of the 6 plates showing the presence of bacteria, 5 contained only single bacterial colonies. The remaining plate showed the pres-

ence of only four colonies. Two hundred and fifty-one broth cultures of muscle in various dilutions, made from the same muscle samples, were found to be sterile after incubation. One broth culture was found to contain bacteria.

Of 126 control plates poured simultaneously, all except 2 plates were sterile after incubation.

Eighty-four plates and 84 tube cultures were made from various dilutions of 30 muscle samples excised from 3 small haddock. After incubation all were found to be sterile. All of 42 control plates were also found to be sterile.

Numerous samples of tissue from the gills, skin and intestines of the 6 haddock showed the presence of bacteria when plated in fish agar after incubation for a week at 20°C.

SECTION II

A second series of bacteriological examinations was made on muscle samples from a group of 8 haddock obtained and stored as described above. As these fish were also to be used for certain bio-chemical studies to be discussed in a later paper, it was necessary to modify the technique used in section I.

Muscle samples of approximately 15 grams were removed from the head, center and tail regions, using aseptic technique. These samples, in sterile containers, were placed in refrigerators, maintained at constant temperatures of 0°, 5°, 10°, 15° and 17°C. All samples, usually 5 in number, from the same fish were incubated at the same temperature. At the end of each 24-hour period one of the 15-gram samples from each fish was removed from storage.

Portions of approximately one gram were cut from each sample at the time that it was taken from the refrigerator. These portions were transferred to sterile nutrient broth tubes. These tubes were incubated at 25°C., and examined for evidence of bacterial growth after incubation. Those showing growth or appearing doubtful were checked by transferring 1 cc. portions to Petri plates, pouring, and examining after suitable incubation.

Of 38 muscle samples examined, only four showed growth, and of these not more than one came from any single fish. The

procedure used in this experiment involved more handling of the samples and therefore more possibility of accidental contamination than was the case in section I.

Transfers of tissue samples from the gills, intestines and skin of this group of haddock showed that all samples made therefrom contained bacteria capable of growing on nutrient agar incubated at 25°C.

SECTION III

Bacteriological examinations were made of the blood from three living fish, two silver hake (*Merluccius bilinearis*), and one squirrel hake (*Urophycis chuss*). The fish were caught off Gloucester harbor. As soon as the fish were taken from the water the ventral portion was wiped with a cloth and 95 per cent alcohol poured over the surface. The alcohol was burned off and a ventral incision was made from the anus to the gills with a pair of sterile scissors. A sterile hypodermic needle was inserted into the beating heart and 0.5 cc. of blood withdrawn. The blood was transferred to a tube containing 4.5 cc. of sterile physiological salt solution.

Several hours later the blood samples in saline were diluted in the laboratory and the various dilutions transferred in 1 cc. amounts to nutrient agar plates and nutrient broth tubes. Half of the cultures were incubated under anaerobic conditions. All were incubated at 25°C., for ninety-six hours.

Due to the small quantities of blood obtained, no dilutions lower than 1:10 were possible. All of the samples from two of the fish were found to be sterile, under both aerobic and anaerobic conditions. Only 1 of the 12 aerobic plates in which blood from the third fish was cultured showed evidence of bacterial growth; the other 11 plates were sterile. This plate had but one bacterial colony.

SUMMARY

One hundred twenty sub-surface muscle samples from 6 different haddock were excised under aseptic conditions and subjected to bacteriological examination. From these samples, 336

aerobic fish-agar plates and 336 aerobic fish-broth tubes were made. Bacteria were found in 6 of the 336 plates, or 1.8 per cent of the plates poured. All of the plates found to contain bacteria were dilution plates and in only one instance were duplicate plates from the same sample. Bacteria were found in 2 of the 126 control plates. Bacteria were found in only one of the 336 tube cultures made from the same samples.

From 38 muscle samples taken aseptically from 8 additional haddock, broth tube transfers were sterile from 34 of the samples and 4 contained bacteria. The methods used in this experiment were such that the possibilities of accidental contamination were more numerous than in the previous experiment.

Evidence was also obtained that the skin, gills and intestines of all the above-mentioned haddock contained bacteria.

Blood removed from the hearts of 3 hake immediately after taking from the water was found to be sterile in all anaerobic and aerobic cultures made from 2 fish. The blood of the third fish was found sterile except for the presence of a single colony on one of the 12 aerobic plates, which was probably an accidental contamination.

The results obtained confirm the findings of Harrison, Perry and Smith (1926), who reported that bacteria were not found in the muscle tissue of freshly-caught haddock, and are in accord with others who have found the flesh of fish to be sterile.

REFERENCES

BROWNE, W. W. 1918 Abstr. Bact., **2**, 6.
BRUNS, H. 1908 Arch. für Hyg., **67**, 209.
FELLERS, C. R. 1926 Univ. Wash. Pub. in Fisheries, 1, No. 8, 157.
GEE, A. H. 1927 Contr. Canad. Biol. Fish., N. S., **3**, No. 12–23, 347.
GEE, A. H. 1930 Contr. Canad. Biol. Fish., N. S., **5**, No. 13–18, 433.
HARRISON, F. C., PERRY, H. M. AND SMITH, P. W. P. 1926 National Research Council of Canada, Rept. 19.
HUNTER, A. C. 1920 Jour. Bact., **5**, 353.
MÜLLER, M. 1903 Arch. für Hyg., **47**, 127.
STEWART, M. M. 1930 Report of the Food Investigation Board. G. B.
ULRICH, S. 1906 Zeit. für Hyg., **53**, 176.