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Serology of cod in Norwegian waters.

Interim report on technique.

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In the study of races and populations of fishes, hereditary characters have become increasingly significant the last few years; and bloodtypes are now commonly used in this kind of investigations.

In 1959 a study conserned with the serology of cod (Gadus morhua) was started at the Institute of Marine Research, Bergen, with identification of cod-populations in Norwegian waters as ultimate purpose. Since 1961 the work has been supported by the Norges Almenvitenskapelige Forskningsråd.

The technique used in the Norwegian cod-studies and described here, is roughly the same as techniques used in other serological work.

Living cod to be studied, are kept in an aquarium. When samples are collected, the individual fish is anaesthetized by immersion in a solution of MSS 222, Sandoz (0,02%) in sea water. With a 60 mm long and 0.70 mm diameter needle, approximately 5 ml blood is drawn from the heart. The fish is then tagged, and returned to the aquarium for later sampling.

50 I.E. heparin per blood-sample prevents clogging.

Serum is washed out with physiological saline solution (11.7 g NaCl/l), and the blood-cells are injected into rabbits, three rabbits for each fish. 0.1 ml cells are injected into one of the external marginal veins of the rabbits' one ear the three first days of four weeks, except the first injection of each of the last three weeks. These injections are set intra-abdominally. Fresh blood for the last six injections is secured by new sampling from the fish.

10 - 14 days after the last injection, 50 - 60 ml blood is drawn from the other ear of each rabbit. The blood is separated, and the serum is heated to 54°C. for 45 min. to destroy elements that will disturb the agglutination-reactions. After this the serum may be stored at -30°C.

The serum is first examined with regard to raise agglutinins by comparing bloodgroup - titrations of the injected cells with serum before and after the injection.

If the rabbits have raised agglutinins in their sera, general agglutinins are first absorbed by a serie of different blood-cells from cod. Some of these may absorb the special agglutinins as well, but in one or more of the sera, special agglutinins will remain. These are tested against the blood-cells that have been used for injection. If high consentrations of special agglutinins have been found by titration of a serum, these agglutinins are isolated in a new serie of absorbtions. The serum may then be used as testserum if the consentration of agglutinins still is high enough.

55 rabbits have now been injected with blood-cells from cod, and ab. 1/3 have given specific positive reactions on agglutinins. From these, 8 different special agglitinins have been isolated.

Temperature, time and medium have been varied to find the most favourable conditions for the reactions. Parallell experiments with tube and slide techniques have also been performed. Generally the classical tube technique seems to give the best results. Room-temperature (20 - 23°C) seems to be optimal, and the reactions can be interpreted with great accuracy after one hour. In some cases, however, the reactions have been stronger if left in a refrigerator overnight. The agglutination is generally stronger in physiological saline solution than in various consentrations of albumin.

The investigation has been complicated by a certain mortality among the cod that is studied, even if this may be partly overcome by storing blood or blood-cells frozen or in glucose/citrat.

In addition to identification of new special agglutinins, the investigation now includes bloodtyping of cod with indentified agglutinins to find antigens of the same system by appliance of Hardy - Weinberg's law of population genetics.