OLFACTORY PERCEPTION IN MIGRATING SALMON

I. L-Serine, a Salmon Repellent in Mammalian Skin

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The presence of a repellent for migrating coho (*Oncorhynchus kisutch*) and spring (*Oncorhynchus tshawytscha*) in the skin of salmon predators has been previously reported (1). The repellent was estimated to be active at a concentration of one part in at least several million parts of water. This is not surprising in view of the known ability of minnows to respond to the presence of a dead member of the species when the active material is present at a concentration of the same order of magnitude. In this case the active substance has not been identified but has been suggested to be a purine or pterin (2, 3).

It has been demonstrated that it is a result of keen olfactory perception that salmon are able to return to the parent stream (4, 6). Catfish (*Ameirus*) and killifish (*Fundulus*) have been shown able to detect food solely by olfactory response (7, 8). Because of the very low threshold of activity, it is most probable that the response of salmon to the repellent in mammalian skin is also elicited by way of the olfactory mechanism (1).

Several synthetic substances were tested for salmon repellent activity, some because they are normal components of skin and some for other reasons, but all were found to be inactive (1). When migrating salmon ascending a fish ladder were exposed to the active substance upstream movement was halted for at least 5 minutes and sometimes considerably longer. The fish displayed an alarm reaction characterized by rapid movements confined to a pool of the ladder and on some occasions the fish retreated to a lower level. The next phase involved a keen alertness during which time slight disturbances in the water which normally evoked no reaction caused immediate response. Finally, the "danger" past, the fish resumed the ascent in a normal manner.

A rinse of human hands has now been shown to contain a repellent for all five species of migrating salmon found in Pacific northwest coho, spring, chum (*Oncorhynchus keta*), sockeye (*Oncorhynchus nerka*), and pink (*Oncorhynchus gorbuscha*). In an effort to find a source of sufficient quantity for identification studies, samples of seal and sea-lion skin were ground in a Waring blendor and

thoroughly extracted with water. In neither case was the extract significantly more active than a thorough rinse of the same area of intact skin. Similarly successive human hand rinses showed diminishing activity. The active repellent is therefore a secretory product possibly associated with sweat. For this reason all work is now being carried out on hand rinses.

Hand rinses were prepared by submersion of the hands for 2 minutes in a convenient quantity of water (*i.e.* 1 liter). Prior to preparing extracts from hair-seal and sea-lion, the skin was freed from fatty tissue and hair. All instruments were thoroughly washed in running water, and rubber gloves were worn by the investigators. The samples were introduced into the water at the fish ladder from waxed containers. Each extract was prepared from 25 gm. of skin and made up to a final volume of 265 ml. Aliquots were taken and the solution introduced into the water entering the top pool of the fish ladder. Testing was carried out at Stamp and Skutz Falls on Vancouver Island as previously described (1). Since satisfactory testing conditions are limited to a brief period in the fall of the year, many additional suspected or known components of skin were tested for activity while the isolation work was proceeding. These included phosphate esters, sugars, fatty acids, purine and pyrimidine compounds, enzymatically hydrolyzed casein, and many others. All compounds were inactive.

To determine the ion exchange properties of the repellent dialyzed washings from human hands were concentrated to *ca.* 50 ml. and passed through beds of resin 1 inch × 12 inches. The bed was then washed with 3 volumes of distilled water and the eluate tested for activity. One of each class of strong acid, weak acid, strong base, and weak base resin was tested. The resins were conditioned to either the hydrogen or hydroxyl form prior to use. The repellent was bound only by strongly acidic and strongly basic resins. The active component of human skin, seal-skin, and sea-lion skin is water-soluble and partitions into the aqueous phase from diethyl ether and *n*-butanol. It is stable to prolonged soxhlet extraction and is not destroyed at pH 2 or 11 on standing 2 days at room temperature. The repellent is not steam volatile nor is it destroyed by this treatment, neither is it volatile at 25 mm. Hg. on a boiling water bath. It is dialyzable. Activity is not destroyed by a wide variety of proteolytic enzymes. Dialyzed concentrates have been stored in the frozen state for many months without loss of activity.

A Shandon continuous paper electrophoresis apparatus equipped with side electrodes was employed to further purify the repellent. A 3 mm. Whatman paper was used and the distance from the wick to the top of the serrations was 12 inches with a paper width of 17 inches between electrodes. The apparatus was operated at 520 to 540 volts at a current of 10 ma. The wick was a $\frac{1}{8}$ inch strip of Whatman 3 mm. paper which entered the paper $\frac{1}{2}$ inch below the top of the electrode and was positioned over tube No. 7. Ten hand rinses were collected in 2 liters of water from the same investigator at 20 minute intervals.

The washings were concentrated to 100 ml. at 40°C. in the flash evaporator and the concentrate was dialyzed for 68 hours against 3 liters of water at +1°C. The dialysate was concentrated to *ca.* 0.25 ml. on a flash evaporator and made up to 5 ml. with 0.4 M acetic acid which was also used as the developing electrolyte. The volume in each of the 30 collecting tubes was approximately 5 ml. The α-amino nitrogen distribution in the contents of the tubes was measured colorimetrically with ninhydrin employing a valine standard (Fig. 1). Total nitrogen in the active fraction, measured with the Nessler reagent, was in general agreement with values obtained by the ninhydrin re-

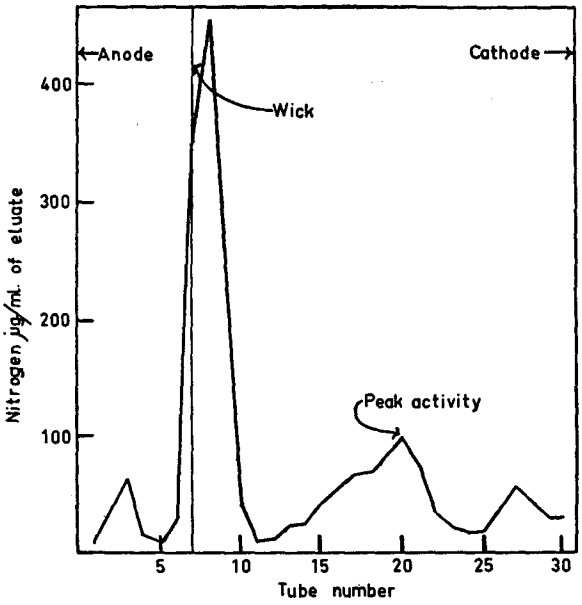

FIG. 1. Continuous paper electrophoretic nitrogen distribution of hand rinses described in text.

agent. Repetition of the experiment resulted in activity occurring in the same fraction and a dry weight determination showed that amino acids accounted for nearly all the total solids in these fractions.

Paper chromatograms were run on the active fraction from continuous paper electrophoresis. Solvent systems used were pyridine-H_2O (acetic acid vapor); *tert.* butanol-formic acid; phenol-water, and butanol–acetic acid–water. Serine was identified as a major component of the active fractions. Four other ninhydrin-positive compounds were present, three of which were identified as valine, leucine (or isoleucine), and cystine. A compound which runs close to serine in several solvents has not yet been identified. In preliminary tests carried out at the fish ladder *l*-serine was active at a dilution similar to the estimated concentration of the natural repellent. The *d*-isomer showed no

activity. The *l*-isomers of the other identified, as well as closely related amino acids, were inactive. Before further tests could be carried out the water levels had become excessive at the fish ladder. A run of coho was located at Sweltzer Creek, near Cultus Lake, British Columbia. The fish were fighting a strong current, and testing conditions were good. The tester, wearing rubber hip boots, stationed himself just upstream from a sandbar which formed an obstruction making it necessary for the salmon to pass through a narrow opening about 2 feet deep. Two observers were stationed several yards downstream. When the fish (usually about 20) reached the constriction the material to be tested was introduced upstream. A hand rinse immediately scattered the salmon back downstream and they returned in *ca.* 5 to 10 minutes. *l*-Cysteine, *l*-leucine, *l*-isoleucine, *l*-valine, *l*-methionine, and *l*-cystine were all inactive when introduced at levels of 10, 5, 1, and 0.5 mg. per 180 ml. of water introduced into the stream. *l*-Serine was tested several times on different days and the results confirmed the finding obtained at the fish ladder. *l*-Serine definitely elicited a typical alarm reaction but the effects were neither so dramatic nor of so long a duration as the response obtained by a hand rinse. However, *l*-serine was active at extreme dilution which we estimate to be of the same order of magnitude as the natural repellent.

The ability of salmon to detect an odour from predators has very obvious survival value. In addition, such a salmon repellent has considerable potential value in deterring salmon from physical barriers and polluted waters. Used in conjunction with attracting stimuli, salmon could be directed to an alternative course around obstacles to migration imposed by increasing industrial developments and uses of natural waters.

Work is continuing in an effort to obtain a purified preparation with as much activity as a hand rinse.

SUMMARY

The properties of a repellent for migrating salmon present in mammalian skin are discussed. *l*-Serine which is present in the washings of human skin has been shown to have repellent activity at extremely high dilution.

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REFERENCES

1. Brett, J. R., and MacKinnon, D., *J. Fisheries Research Board Canada*, 1953, **11**, 310.
2. Von Frisch, K., *Naturwissenschaften*, 1941, **29**, 321.
3. Huttel, R., *Naturwissenschaften*, 1944, **29**, 333.
4. Hasler, A. D., and Wisby, W. J., *Am. Naturalist*, 1951, **85**, 223.
5. Hasler, A. D., *J. Fisheries Research Board, Canada*, 1954, **11**, 107.
6. Wisby, W. J., and Hasler, A. D., *J. Fisheries Research Board, Canada*, 1954, **11**, 472.
7. Parker, G. H., *J. Exp. Zool.*, 1910, **8**, 535.
8. Parker, G. H., *J. Exp. Zool.*, 1911, **10**, 1.