

Brief Communication

METHODOLOGICAL PROBLEMS IN WHOLE-BODY
AUTORADIOGRAPHY OF RAINBOW TROUT

Ullbergs method for whole-body autoradiography (Ullberg 1954) has been applied to study the distribution of drugs in rainbow trout (Bergsjø *et al.* 1979). During the technical procedure several problems occurred.

Administration

The short, wide oesophagus entails easy regurgitation of stomach contents. The fish were carefully watched for 15 min following administration and no case of regurgitation was observed. During sectioning, however, a few cases were seen of partial regurgitation of the capsule into the cranial part of the oesophagus. Furthermore, on developing the films, a diffuse darkening in the throat/gill region was observed in several cases resulting from vomiting of stomach contents containing isotope-labelled material (Fig. 1). This phenomenon complicated assessment of the degree of radioactivity in the gills and led to uncertainty as regards dose levels. To avoid these problems one might try to administer the test substance in a pastelike preparation.

Mounting and freezing

A special feature of the anatomy of the rainbow trout is the location of the gall bladder in relation to the liver. The gall bladder is located in the right side of the fish displacing the liver to the left, the degree of displacement depending on the fullness of the former. Mounting the fish with its left side facing upwards resulted in great loss of liver tissue when trimming down to a level which enabled satisfactory sectioning of the gall bladder and vice versa. This feature had to be taken into consideration when mounting the fish in order to minimize loss of organ material required for further analyses.

Cryosectioning

Stones liable to damaging the edge of the microtome knife were occasionally present in the gastrointestinal tract. Gelatine

capsules containing some of those were administered to a number of fish which were x-rayed under benzocaine anaesthesia (Fig. 2). It was found that the stones having caused problems during the experiment, gave a satisfactory contrast on x-ray. Thus, by x-raying or screening the fish to be used, those containing damaging stones can be excluded. Alternatively fish cultivated in pools free from foreign bodies could be used.

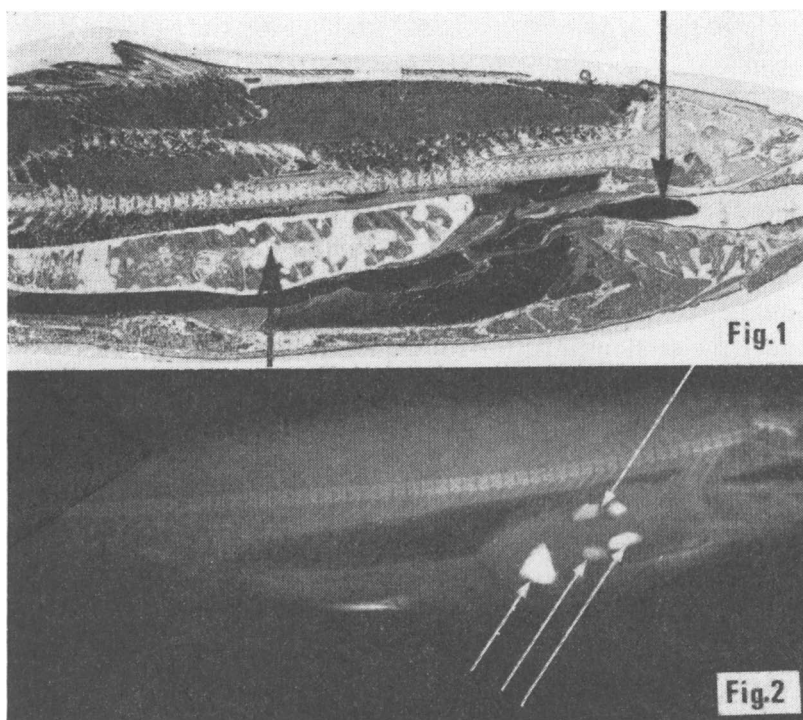


Figure 1. Autoradiogram of a fish 48 h after oral administration of a ^{14}C -labelled compound. Dorsal arrow indicates blackening in the throat region. Ventral arrow indicates artificial blackening in the region of the swim bladder.

Figure 2. X-ray photograph of a fish after oral administration of a gelatine capsule containing 2 stones. Dorsal arrow indicates the capsule. Ventral arrows indicate 3 stones present beforehand.

Abdominal adipose tissue in rainbow trout consists largely of polyunsaturated fatty acids with a low freezing point, a fact which made satisfactory sectioning difficult even at -20°C . Adipose tissue tended to stick to the microtome knife resulting in contamination with isotope containing material which in turn

could be transferred to other anatomical structures. The problem was solved by wiping off the knife with ethanol after each sectioning. Lowering the temperature in the cryostat could reduce this problem.

Parts of the dorsal muscle tissue attached badly to the tape. A better attachment was obtained if, during trimming down to the desired level, a section thickness of less than 50 μm was employed in order to reduce the extent to which mechanical crushing occurred.

Development

A blackening of the region of the developed film representing the swim bladder often occurred (Fig. 1). Since no material is attached to the tape in this region, the tape will in consequence adhere strongly to the film resulting in electrostatic discharge on separating the two. In general, this problem may be reduced by unstripping the tape in an atmosphere with high relative humidity or under water. Unstripping under water may, however, render the section unfit for subsequent staining. In this very case the problem may easily be solved by filling the swim bladder with mounting medium before sectioning.

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