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IODINE ACCUMULATION IN A NEMERTINE, *LINEUS RUBER*

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INTRODUCTION

In an article on the comparative morphology and physiology of the vertebrate thyroid gland in *Comparative Endocrinology* (Gorbman, 1959), the author shows a diagram indicating the localization of the iodoproteins and related compounds in the tissues of various types of vertebrates and also in two hypothetical invertebrates. The two latter are intended to show that in the invertebrates the process of uptake and metabolism of iodine probably evolved from rather feeble and generalized processes to one of considerable activity localized especially in the pharynx. At the time, no actual creature was known which showed this specialization. On the other hand Hubrecht in 1883 had put forward the idea that the vertebrates may have had their origin in some nemertine-like creature, and this idea has been recently renewed, independently and on quite different grounds, by Jensen (1960) and by one of the authors of this paper (Willmer, 1960). If this were their origin then the pharynx of the nemertine would clearly be worth examining for its possible role in iodine segregation. The nemertines might thus, if they were found to pick up iodine, fill the role assigned by Gorbman to his hypothetical invertebrate, and an investigation of their ability or inability to pick up radioactive iodine would obviously put the matter to the test.

METHODS

Specimens of *Lineus ruber* were obtained from the Marine Biological Laboratory, Plymouth, and kept in a bowl of sea water constantly renewed by circulation from the aquarium in the Cambridge Physiological Laboratory. The bottom of the bowl was covered with a thin layer of sand and several flat stones were provided so that the worms could retreat from the light. It was found to be important to keep the worms cool and in a dim light.

For experimental purposes the worms were transferred for at least 24 hr. and usually 48 hr. to iodine-free artificial sea water of the standard composition shown in Table 1. During this time they were again kept cool and in the dark.

Radioactive iodide, ^{131}I (Radiochemical Centre IBS 3) carrier-free, was added to artificial sea water, to artificial sea water that was diluted with de-ionized water, to artificial sea water to which sodium chloride had been added, or to artificial sea water which had an osmotically equivalent amount of sucrose added. The concentration of radioactive iodide was in each case 5 μC./100 ml. of sea water. The worms were placed individually in glass pots with loose-fitting caps; each pot contained 25 ml. of the appropriate radioactive solution.

For most purposes ^{131}I was used, but for autoradiography with AR 10 stripping film (Kodak Ltd) ^{125}I was used on account of its better localization. For histological purposes tissues were fixed with Susa fixative generally directly but sometimes after the worm had been anaesthetized with a solution of 7·5% $MgCl_2$ diluted with an equal volume of sea water. Several staining methods were used, but Masson's trichrome was found to be most useful for general purposes and Haemalum for the autoradiographs, though the latter stain was not very successful, since it stained the gelatine base of the film as well.

Table 1

	g./l.
NaCl	23·427
KCl	0·729
$CaCl_2.6H_2O$	2·218
$MgCl_2.6H_2O$	10·702
$Na_2SO_4.10H_2O$	8·967
$NaHCO_3$	0·210

For measuring the radioactivity, the worms were transferred by means of a small glass hook to non-radioactive sea water for 2 min. or so. This process was repeated twice before the worm was finally transferred to a weighed plastic pot. After weighing, the plastic pot was placed in the well of a thallium-activated sodium iodide crystal and the radioactivity was determined by means of a conventional scintillation counter and scaler. The radioactivity was expressed as counts per mg. of worm per second divided by the counts per mg. of sea water per second. In most experiments the results from about six worms in each medium were pooled.

RESULTS

The first series of experiments established the fact that *Lineus ruber* showed radioactivity which after 48 hr. was on the average some twenty times that of an equivalent weight of the sea water in which it was immersed. There was, however, very considerable variation from worm to worm, and some specimens hardly concentrated the iodide at all while others collected far more than the average. The reason for this variability has not so far been established. In one or two cases the worms which showed no uptake were found to be unhealthy or moribund.

By dividing the worms into three segments, pre-oral, pharyngeal and intestinal, it was found that the first two of these segments showed far more concentration than the whole of the intestinal segment although the latter might be many times the weight of the other two. The distribution of the radioactivity in the whole worm is shown in Fig. 1 (Pl. 1) which was obtained in the following way. A whole worm was placed in artificial sea water containing ^{131}I for 48 hr. It was then washed with non-radioactive

Fig. 1. (Plate 1). Autoradiograph from *Lineus ruber* subjected to ^{131}I for 48 hr. The head of the worm is to the left, and the radioactivity is clearly greatest in the region of the mouth but is heavy throughout the whole pharyngeal region.

Fig. 2. (Plate 1). Autoradiograph of a section of the pharyngeal region of *Lineus ruber* kept in ^{125}I for 48 hr. There is some radioactivity in the skin but most of it is concentrated in the pharyngeal epithelium and underlying glandular tissue. The proboscis which appears just above the pharynx is negative. Exposure, 8 days. Paraffin section.

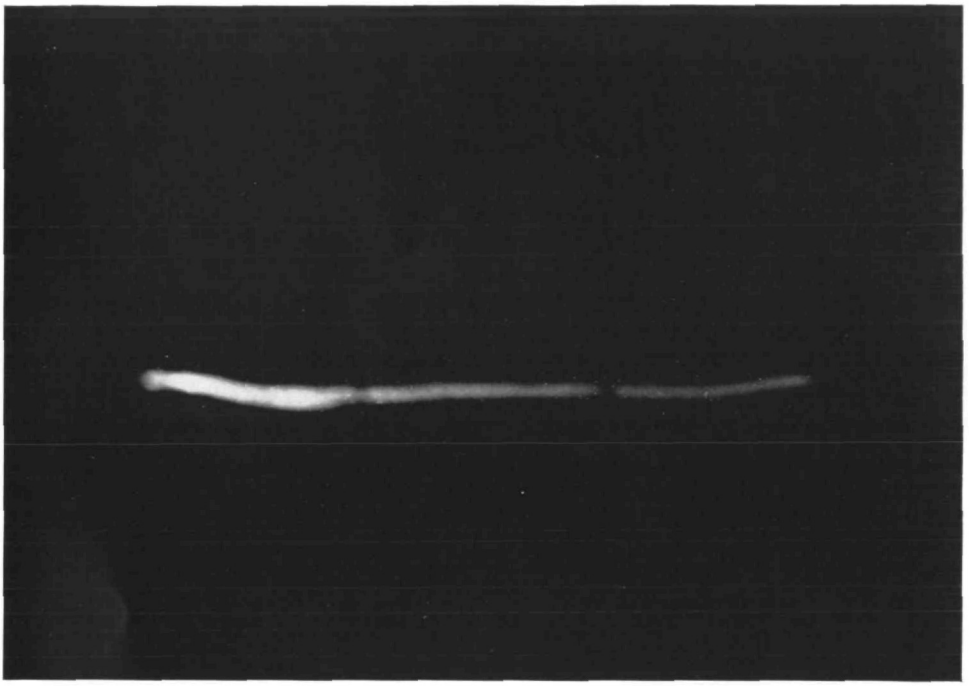

Fig. 1

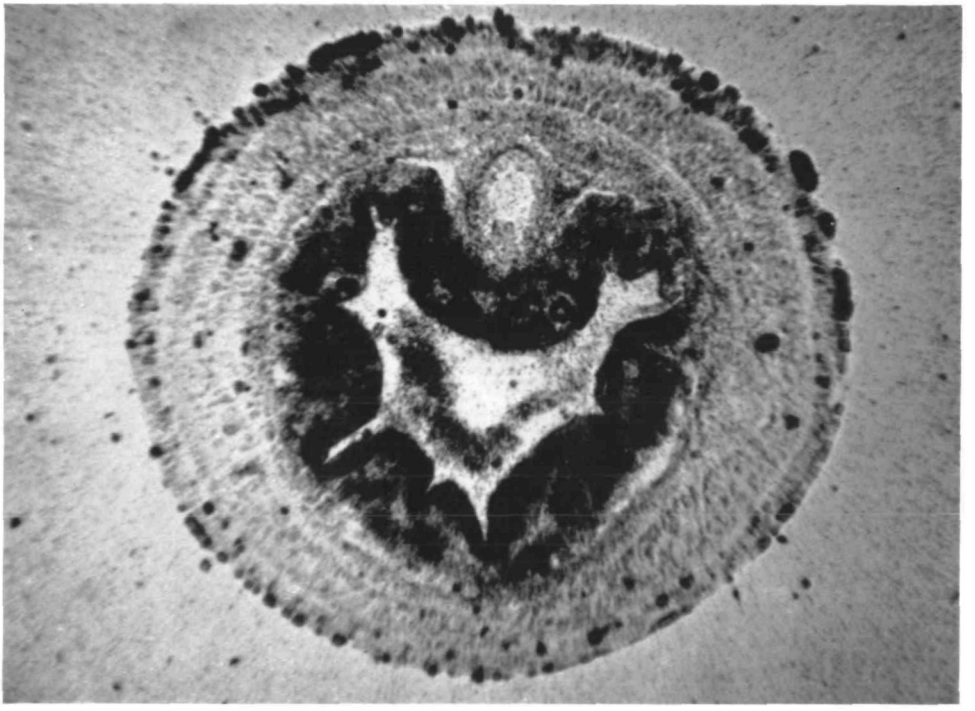

Fig. 2

sea water, anaesthetized with $MgCl_2$ solution and then fixed in the extended condition on a glass plate in formalin vapour. It was then dried in an oven at about 90° C. and, when thoroughly dry, clamped against the emulsion of a photographic plate for 2 hr. The resulting radio-autograph was then developed and printed. The greatest concentration is clearly in the anterior region around the mouth and upper part of the pharynx, though the whole pharyngeal region is strongly radioactive. When serial sections of shorter segments of worm were examined it was found that the concentration of radioactivity coincided rather closely to the amount of pharyngeal tissue present and this was confirmed by a series of autoradiographs obtained from serial sections of which one is illustrated in Fig. 2 (Pl. 1). The autoradiographs were not entirely satisfactory, since they were variable in the results which they gave. To some extent, they may have been dependent on the degree of contact between the film and the section and in one or two cases failure to dry the sections completely may have caused some spurious reduction of the film. Another more probable reason for this variability will, however, be discussed later. The pharyngeal epithelium is primarily ciliated, but backing this layer of ciliated cells are several layers of both eosinophil and basophil epithelial cells constituting a thick glandular layer. Some of the basophil cells connect to the surface by sending up processes between the ciliated cells. In the autoradiographs all the layers seem to be involved, but more work is necessary to follow the movement of iodide into and out of the pharynx. As predicted by Gorbman the mucous materials in the pharynx and intestine were also found to be radioactive.

When worms were made radioactive by treatment for 2 or 3 days in iodinated artificial sea water and then transferred to non-radioactive sea water it was found that the worms maintained their radioactivity unimpaired, when isotope decay was taken into account, for several days. When autoradiographs were made from a worm which had been in radioactive sea water for over a week and then artificial sea water for about 10 days, it was found that there was then practically no radioactivity detectable in the pharynx but quite a lot in the subcutaneous tissues, particularly in the anterior end of the worm. This somewhat anomalous finding clearly raises the question of what does the animal do with the iodide it has picked up. From the autoradiographs it would seem probable that free iodide is picked up and then slowly attached to mucoprotein and passed into the gut where some of it may get absorbed and distributed to the subcutaneous tissues.

The autoradiographs, of course, do not show whether there is any true 'thyroid' function. To attempt to answer this question several worms were exposed to radioactive sea water and then the head and pharyngeal regions were taken, homogenized in a Tris buffer and digested with trypsin. Samples of the digest were chromatographed and the radioactivity of the paper strip was determined. By far the largest peak of radioactivity was found in the iodide position, but only a very small amount of radioactivity was found in the 'thyronine' position. Curiously enough no radioactivity was found either at the origin or in the 'tyrosine' positions. These observations may perhaps explain some of the anomalous autoradiography in the following way. Although the living worm has been shown to retain its iodine quite tenaciously, the dead worm after fixation could behave very differently and the free iodide could easily leach out to a greater or less extent during the various manipulations of the tissues. This may

account for some of the variability of the autoradiographs and further experiments on these lines are necessary before the cellular sites of uptake and iodine metabolism can be established.

Experiments directed towards the solution of other problems of nemertine physiology had shown that the histology of the pharynx changes very noticeably in different concentrations of sea water, and it was therefore of some interest to see if this in any way affected the uptake of iodide. For example, it might be thought that if the concentration of chloride in the sea water were reduced iodide might be taken up in its

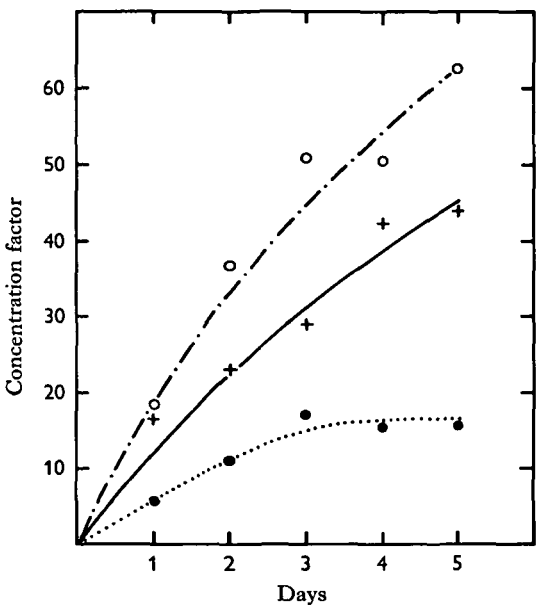

Fig. 3. Uptake of ^{131}I by worms maintained in +—+ artificial sea water; ●·····● artificial sea water diluted with an equal quantity of distilled water; O—·—·O artificial sea water + 1 g. NaCl (in one experiment 1·5 g. NaCl was used) per 100 ml.

$$\text{Concentration factor} = \frac{\text{counts/mg./sec. of worm}}{\text{counts/mg./sec. of sea water}}.$$

The points for the first 3 days represent the mean results for more than fifteen worms in each medium. For days 4 and 5 the points represent the means for eight worms in each case.

place. Conversely, less iodide might be collected in the presence of an excess of chloride in the water. When, however, experiments were made along these lines the iodide uptake did indeed vary with the concentration, but in such a way that the more concentrated the sea water the more iodide was picked up, even though the concentration of the iodide in the final medium was always identical (see Fig. 3). In dilute sea water the ciliated epithelium of the pharynx is prominent and the underlying accessory cells are 'emaciated'; while when the NaCl concentration is raised the basophil and eosinophil cells are turgid and rounded while the ciliated cells are generally in poorer shape. In even higher concentrations (i.e. sea water containing more than an extra 2% NaCl), the ciliated cells may actually round off and slough away. It is thus possible that the collection of iodide may depend on a pinocytotic process in the more glandular

cells. The amount of thyronine was also found to be rather greater in animals exposed to concentrated sea water, again, suggesting that the glandular cells are involved. The effects on the iodide uptake are probably dependent on the NaCl content itself, since making up the osmotic pressure of dilute (half-strength) sea water to that of normal sea water by the addition of sucrose (20·5 g./100 ml.) did not significantly change the iodide uptake from that in dilute sea water (Fig. 4). On the other hand, some preliminary experiments showed that treatment of the worms with 10^{-4} M ouabain depressed the iodide uptake, but it also depressed the activity of the worm as a whole and the results may thus not necessarily mean that the iodide-concentrating mechanism had been specifically inhibited.

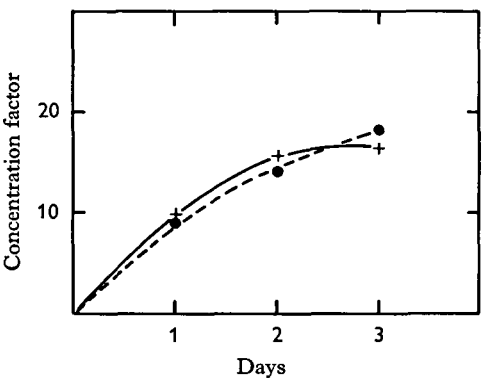

Fig. 4. The result of a single experiment in which the osmotic pressure of the diluted sea water was made up to that of normal sea water by the addition of sucrose (20·5 g./100 ml.). The uptake of iodine was no greater than in the dilute sea water. Ordinates as in Fig. 3. ●---● Dilute sea water; +—+ dilute sea water plus sucrose.

DISCUSSION

These somewhat exploratory results seem to be of interest on several counts. First, they demonstrate an uptake of iodine specifically related to the pharyngeal region of the nemertine worm and this property exactly fulfils the requirement for a precursor of thyroid activity in relation to possible vertebrate ancestry. Secondly, the 'thyroid process', if such it be, is still in an elementary form in the nemertine and is little more than that of iodide accumulation, though an ability to form a little thyronine is also certainly present. Clearly an uptake of iodide is the first essential step in thyroid function, and while it is not by any means uniquely present in the nemertine pharynx, the extent of the uptake is quite remarkable. In the hemichordate *Saccoglossus* bound iodine as well as free iodide can be demonstrated in extracts of its tissues (Barrington & Thorpe, 1963) in the form of monoiodotyrosine, but no thyronines were detected. In the urochordates, triiodothyronine and thyroxine are both believed to be present (Barrington, 1965). Even in some vertebrates thyroxine may be found only in minimal amounts (Gorbman, 1959). Thirdly, the complex histological and cytological character of the pharyngeal region of the nemertean is consistent with its participation in the formation of the thyroid in the course of evolution; it is also of particular interest that some of the cells in the pharynx have cytological features in common with the so-called chloride secretory cells of the gills of fishes and the oxyntic cells of the stomach in higher forms. Fourthly, the concentration of iodide is

greater under conditions of high ambient chloride concentration and this behaviour is paralleled in the thyroids of turtles when these animals are starved of water. In these vertebrates the uptake of iodide may continue by drawing on that which has passed to the urinary bladder (Shellaburger *et al.*, 1956). Fifthly, the nemertine worm is an easily accessible creature capable of living for many days in sea-water media and is therefore a potentially important creature from the point of view of investigating the first crucial step in thyroid function, namely the uptake of iodide. It is well known that in many vertebrates thyroid function changes coincidentally with migrations to other environments differing in salinity or temperature, and thus a study of the nemertine might be able to clarify some of the problems connected with these phenomena, since it is now clear that changes in its ability to accumulate iodide occur in response to salinity changes and it is also a creature that is very sensitive to temperature changes. The physiological significance to be attached to iodide accumulation, to the formation of mono- and diiodotyrosine, triiodothyronine and thyroxine and their utilization and/or excretion could well receive elucidation by reference to the relatively simple events in the nemertine.

SUMMARY

1. Studies with radioactive tracers show that the nemertine *Lineus ruber* actively accumulates iodide in its pharynx, and stores it mostly as iodide.

2. When the sodium chloride concentration of the ambient sea water is raised, the amount of iodide accumulated is increased, and traces of a 'thyronine' can be detected.

3. When the osmotic pressure was raised with sucrose (instead of NaCl) there was no increased uptake of iodine.

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