XIII. ON THE OCCURRENCE OF MANGANESE IN THE TUBE AND TISSUES OF *MESOCHAETOPTERUS TAYLORI*, POTTS, AND IN THE TUBE OF *CHAETOPTERUS VARIOPEDATUS*, RENIER.

BY CYRIL BERKELEY.

From the Marine Biological Station, Nanaimo, B.C.

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A NUMBER of marine annelids excrete a mucous substance which hardens to form a tube in which they live. Many of these tubes are partially soluble in cold dilute alkaline solutions.

In the course of an attempt to determine the nature of the material thus dissolved from the tube of an annelid which occurs commonly on the British Columbia coast, *Mesochaetopterus Taylori*, it was found that the substance precipitated on acidifying the alkaline extract yielded a bright blue ash on burning. Qualitative tests showed that this was due to manganese and led to the examination of the tube material and the tissues of the animal for that element. Preliminary quantitative tests indicated that it was present in both cases in unexpectedly large amounts and more careful determinations seemed warranted.

Traces of manganese are known to occur very generally in animal tissues and Bradley [1910] has shown that the element is normally present in considerable amounts in the tissues of fresh-water mussels. It was also observed by that author that the nacre of the shells of both living and fossil Unionidae is rich in manganese and this, so far as the writer is aware, is the only case of its occurrence in quantity in an animal secretion at all comparable with the tube of a worm.

All the more recent methods for the determination of manganese in organic material depend upon the extraction of the manganese salt from the ash and its oxidation to permanganic acid, which is estimated colorimetrically or by titration with arsenious acid or an arsenite. Reiman and Minot [1920] give an excellent summary of the literature of the subject and describe a method for determining the very small amounts of manganese occurring in human blood and tissues. In the present case the amount of manganese present was comparatively so large that the somewhat elaborate method of ashing the material and leaching the ash described by these authors was unnecessary. In working with the tube material it was, indeed, not found necessary to use

any oxidising agent to complete the destruction of the organic matter as is recommended by Bradley. Direct burning at a moderate temperature produced an ash, which, excepting some sand, was completely soluble in 35 % nitric acid, to which a few drops of hydrogen peroxide were added. Subsequent fusion of the undissolved residue with potassium nitrate rendered no more manganese soluble. In the case of the tissues the addition of a small quantity of potassium nitrate was found necessary. With this modification and substitution of a solution of sodium arsenite for that of arsenious acid for titration of the permanganic acid, the method used has been substantially that of Bradley. The details of procedure have been as follows:

0.2 to 1.0 g. of material is weighed into a porcelain crucible. The bulk of the organic matter is burnt off at a low temperature. Potassium nitrate is then cautiously added if necessary. The crucible is then gradually brought to a bright red heat and maintained there for about half-an-hour. When cool 2 cc. of 35 % nitric acid and 5 drops of hydrogen peroxide are added to the contents of the crucible which is cautiously heated over a small flame. The acid is then diluted and decanted from the small amount of sand remaining undissolved, and the latter is repeatedly leached with small quantities of hot water until the combined leachings amount to about 50 cc. 0.5 cc. of 10 % silver nitrate is added to the solution which is then brought to the boiling point. The vessel is removed from the flame and 10 cc. of 20 % ammonium persulphate cautiously added. The permanganic acid colour develops immediately. The solution is then boiled gently for five minutes and cooled in running water. It is then titrated with a very dilute solution (not stronger than 0.1 g. per litre) of sodium arsenite which has been previously standardised against a solution of pure potassium permanganate reduced by sulphurous acid. An aliquot of this reduced permanganate solution is put through the foregoing oxidation procedure immediately before the sodium arsenite solution is standardised.

In application to aliquots of the standard permanganate solution evaporated to dryness this method gave results of a high degree of accuracy for quantities of manganese as small as 0.00001 g. It is important to boil the solution sufficiently long after addition of the ammonium persulphate to decompose the excess, otherwise too high readings are obtained. The presence of chlorides is stated by some authors to affect the accuracy of determinations of manganese depending upon the principle involved in this method. This has not proved to be the case in the work to be described. Traces of chloride were invariably indicated by the precipitation of silver chloride on adding the silver nitrate to the solution under analysis, but this dissolved on treating with ammonium persulphate and did not affect the determination. No appreciable difference in result was obtained whether the ash were heated with sulphuric acid, to drive off hydrochloric acid, before analysis or not. The figures recorded in Table I illustrate this point and indicate the degree of accuracy attained in duplicate samples.

Table I. *M. Taylora*.

Material	Weight taken g.	Manganese found %	Treatment
Tube	0.1592	0.0276	Ash not treated with sulphuric acid
"	0.3426	0.0305	"
"	0.2000	0.0300	Ash treated " with sulphuric acid
"	0.2176	0.0253	"
Posterior region	1.0690	0.0015	Ash not treated with sulphuric acid
" "	0.7098	0.0015	Ash treated with sulphuric acid

Closer agreement than is indicated could not be expected owing to the difficulty of obtaining uniform samples. The material of the tube does not lend itself to very fine grinding and, as is subsequently shown (Table III), the different regions of the tube differ considerably in manganese content, so that such fluctuations as appear may easily be due to the regions being represented in varying proportion in the samples.

Determinations in individual tubes of Mesochaetopterus Taylora.

It is extremely difficult to dig up the tube of *M. Taylora* entire. It usually runs down more or less vertically through the fine sand for one to two feet and then takes abrupt turns in a more horizontal direction through the coarser material underneath. Almost invariably, therefore, unless very special care is taken, the tube is cut off short of the closed end. The tubes of which the analyses are given in Table II were the longest which could be selected from a large number dug, but only in the case of No. 1 was the terminal portion present. They were freed from adherent sand as far as possible, but varying quantities remained imbedded in the tube material. This was determined by weighing the residue after the ash had been completely extracted with nitric acid and water.

Table II. Tube of *M. Taylora*.

Serial number	Weight of material g.	Sand %	Manganese found mg.	Manganese in material %	Manganese in material - sand %
1	0.8402	45.9	0.297	0.0353	0.0654
2	0.3209	18.4	0.0715	0.0223	0.0273
3	0.2600	13.9	0.0132	0.0508	0.0589
4	0.2248	15.7	0.0385	0.0171	0.0203
5	0.1427	22.0	0.0495	0.0347	0.0445

The results show a considerable variation. It is noteworthy that although No. 3 gives the highest result on the entire material, this is in part due to its low content of sand and that, if calculation be made on the material minus sand, No. 1, in which the largest proportion of the basal end of the tube was present, gives the highest figure. This suggested that the basal end of the tube might be richer in manganese than the rest and that the variation in results was to some extent due to the varying proportion of this end of the tube represented in the material analysed.

Determinations in various regions of the tube of M. Taylora.

The tube can be easily divided transversely into three distinct regions corresponding roughly to the anterior, median and posterior portions of the animal. The uppermost, most of which projects above the surface of the sand bed, is composed of very thin papery material and is almost white. In the median region the wall is thicker and pale brown, whilst that of the posterior region is comparatively thick and frequently two or three layered. It is much darker in colour than the rest of the tube and often contains thickenings where the tube has been broken and repaired. Material from each of these regions was separated from a number of tubes and duplicate determinations made from the bulked samples. The results are given in Table III.

Table III. Regions of tube of *M. Taylora*.

Region	Weight of material g.	Ash %	Manganese found mg.	Manganese in material %	Manganese in material - ash %
Anterior	0.2520	49.2	0.011	0.0043	0.0086
"	0.2126	46.4	0.011	0.0051	0.0096
Median	0.2746	39.9	0.0265	0.0096	0.0160
"	0.3584	36.7	0.0385	0.0107	0.0169
Posterior	0.3426	26.7	0.1199	0.035	0.0475
"	0.4250	27.2	0.1375	0.0326	0.0444

The duplicate samples agree as well as could be expected having regard to the difficulty of drawing uniform samples of material. The results show a marked difference in the three regions, the posterior being the highest and the anterior the lowest. The ash content varies in the inverse sense. The greater part of the ash was sand, but separate determinations of sand were not made in these cases. Calculated to the material minus ash there is still a very large difference between the manganese content of the three regions. There is no doubt this accounts in some measure for the irregularities shown in the determinations in individual tubes (Table II) since, as has been pointed out, these were not complete and it was in the proportion of material drawn from the posterior portion of the tube that they chiefly differed.

Determinations in tissues of M. Taylora.

The question whether the tissues of the worm itself contain manganese in notable quantity arises naturally out of its occurrence in the tube. The body of the worm is divided transversely into three distinct regions like the tube. For analysis these three regions were treated separately. They were removed from a large number of worms and kept in alcohol until hardened. The material was then dried, ground as finely as possible and duplicate samples from each region taken for analysis. The results are given in Table IV.

Table IV. Tissues of *M. Taylori*.

Region	Weight of material g.	Manganese found mg.	Manganese in material %
Anterior	0.7067	0.044	0.0062
"	0.7653	0.0495	0.0065
Median	0.4985	0.077	0.015
"	0.4493	0.0385	0.0086*
Posterior	1.069	0.016	0.0015
"	0.7098	0.011	0.0016

* This result is certainly too low. No potassium nitrate was used in ashing and combustion was therefore not complete.

Manganese is present throughout the body of the worm, but in smaller proportion than in the tube. It is interesting to note that the median portion of the body contains most manganese since the chief glands which secrete the mucous material from which the tube is constructed are situated in this region.

Determinations in tube of Chaetopterus variopedatus.

C. variopedatus is the best known member of the family Chaetopteridae, but does not occur on our coast. A few tubes of this worm were obtained from Woods Hole, Massachusetts, for comparison with those of *M. Taylori* in respect of manganese content. The tube is a great deal wider in relation to the size of the inhabitant than in the case of the latter species and has both ends open. These both protrude above the surface of the muddy sand in which the animal lives. With the exception of these small and narrowed ends the material of the tube is very similar to the posterior portion of that of *M. Taylori*. It was found possible to divide the tube material longitudinally into two layers and it seemed of interest to determine manganese in the two layers separately. The inner one was smooth and clean and of a somewhat darker colour than the outer which was rough and heavily coated with sandy mud. This was cleaned as carefully as possible and the two lots of material dried separately. The results are given in Table V.

Table V. Tube of *C. variopedatus*.

	Weight of material g.	Ash %	Manganese found mg.	Manganese in material %	Manganese in material - ash %
Inner layer	0.3278	24.95	1.501	0.457	0.610
" "	0.3042	28.27	1.314	0.431	0.602
Outer layer	0.2806	49.14	0.66	0.235	0.462
" "	0.3021	45.81	0.676	0.224	0.410

The tube of *C. variopedatus* is thus more than ten times as rich in manganese as even the posterior portion of that of *M. Taylori*. The inner layer contains about twice as much as the outer one, but this is in part due to there being more sand in the latter. Expressed on ash-free material the ratio is about 3 : 2. This, coupled with the observation that manganese occurs in comparable quantity in the tissues of the allied species *M. Taylori*, suggests very strongly that the manganese in the tube is secreted by the worm and is

not due to the direct action of sand or sea water on it. It is probable, on the contrary, that these agencies tend to remove manganese from the outer layers of the tube since the sand lining the cavity from which a tube has been dug is invariably stained dark brown and looks precisely as if a rusty iron bar had been withdrawn. This rustiness is almost certainly due to oxides of iron and manganese resulting from the decomposition and wearing of the outside of the tube by sand and water.

In every case in which the test has been made iron has been found accompanying manganese in the ashes of the materials dealt with in this paper.

Examination of tubes of other worms for manganese.

The tubes of three other worms which occur commonly in this neighbourhood were examined for manganese; those of two *Sabellids* and a *Spiochaetopterus*. It was present in just sufficient quantity to be determined in the material from one of the sabellid tubes. No trace could be detected in that of the other sabellid or of the spiochaetopterus using quantities of material up to 0.75 g. The last case is particularly interesting because, not only is the animal nearly allied genetically to the chaetopterids in the tubes of which manganese occurs, but also it is always found in the sand beds living immediately alongside *M. Taylora*. We seem to have here a very marked instance of a physiological divergence in species taxonomically and ecologically similar.

DISCUSSION.

As an outcome of the observations recorded the question of the source and function of manganese in the tubes of worms naturally arises. It is fairly clear, for reasons which have already been advanced, that the manganese arrives in the tube from the worm outwards and not from the sea water or sand inwards. Whilst it is conceivable that the worms concentrate manganese from the sea water direct it is not probable, because the quantity of the element in sea water is relatively very small indeed and the worms do not pass large quantities of water through their bodies, but strain their food material out of it. Enders [1909], who has carried out a detailed study of the life history and habits of *C. variopedatus*, holds that, in the case of that species, any sand which is suspended in the water taken by the worm into its tube is also discarded and that only diatoms and small animals are taken into the digestive tract. This does not, however, seem to be the case with *M. Taylora*, since a considerable quantity of sand was found in the posterior region of the animal. A sample of the diatoms which are normally to be found in the water covering the sand beds in which this worm lives, and which probably constitute a large part of its food, was obtained by dragging a fine net over beds of eel-grass growing in the immediate neighbourhood. The diatoms were separated by fractional sedimentation from the small animals and debris accompanying them, dried at 100° and ash and manganese determined. 60.8 % of

ash containing 0.007 % of manganese was found. The manganese was all readily extractable from the ash by 35 % nitric acid containing hydrogen peroxide. No further manganese could be detected after fusing the residual silica with sodium carbonate, so that it appears to be contained in the organic portion of the diatom. Here, therefore, we have an immediate source of manganese readily available to the worm. It is, however, not impossible that it is also derived from the sand which, in the case of *M. Taylori*, undoubtedly passes through the digestive tract in considerable quantity.

Manganese was determined in the sand in which the worms were living by sodium carbonate fusion followed by application of the method previously used, and 0.158 % found present. About a fifth of this was directly soluble in 35 % nitric acid and was therefore not in silicate combination. The sand passed through the animal therefore provides an ample source of manganese and its utilisation does not necessitate the assumption of any power of silicate digestion.

The presence of manganese in organisms has generally been associated with respiration. No evidence is at hand to show that it is to be connected with this activity in worms. It is difficult to see how its presence in the tube can have any connection with respiration, and, in the absence of any evidence to the contrary, it seems most rational to assume that its presence there is of no physiological significance, but is due to the worm excreting its superfluous manganese with the tube-building material, a theory which is supported by the observation of manganese in larger quantity in the lining than in the outer layers of the tube of *C. variopedatus*, and in the mucous secreting region of the body of *M. Taylori*, more than in the other regions. The presence of more manganese in the posterior than in the other portions of the tube of the latter species is also explained on this assumption since this is the oldest part of the tube and has been most frequently relined and repaired. Bradley [1910] has shown that the excreta of fresh-water mussels living on their normal manganiferous diet contain the element in about the same proportion as it occurs in their food, so that a state of manganese balance is maintained—only if they are deprived of manganiferous food is the metal definitely retained by the animal. It is likely that a similar state of affairs exists in the case of worms having manganiferous tubes, but that some of the superfluous manganese passes into the tube material. The deposition of manganese in the nacre of the shell of the Unionidae observed by Bradley is no doubt a parallel case.

SUMMARY.

1. Manganese occurs throughout the tube of *M. Taylori*, but in much greater quantity in the posterior portion than in the other parts.
2. Manganese is present throughout the body of *M. Taylori*, but in smaller quantity than in the tube. The median region of the body, in which the chief mucous secreting glands are situated is richest in manganese.

3. Manganese occurs in far larger quantity in the tubes of *C. variopedatus* than in those of *M. Taylori*. The lining of the tube contains much more manganese than the outer layer.

4. The tubes of local species of *Sabellid* and *Spiochaetopterus* contain no manganese.

5. Manganese is probably present in worm tubes as a waste material excreted by the worm with its tube building substance. It may be derived either from diatomaceous food or from sand passing through the digestive tract with the food, or from both.

REFERENCES.

- Bradley (1910). *J. Biol. Chem.* **13**, 237.
Enders (1909). *J. Morphology*, **20**, 479.
Reiman and Minot (1920). *J. Biol. Chem.* **42**, 329.