Letters to the Editor.

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Spectrographic Analysis of Animal Tissues.

IN NATURE of April 20, 1929 (**123,** p. 601), one of us (H. R.) described a method of spectrographic analysis of mineral and organic substances and pointed out the value of the method for biological purposes. Since then we have undertaken a survey of the animal kingdom with the purpose of detecting and analysing quantitatively those of the less common elements contained in protoplasm and its products which can be investigated by this method.

The animals, if small, are minced whole, or if sufficiently large are dissected and the separate organs minced. The material is then dried at 100° C., powdered in an agate mortar, and a weighed quantity (50 mgm.) taken and rolled in ashless filter paper. The roll is burnt in an oxy-coal gas flame in front of the slit of a quartz spectrograph, the image of the flame being focused on the slit by a quartz lens. The spectrum is recorded on a photographic plate. In the case of liquids, such as blood, 0·1 c.c. is pipetted on to a rolled filter paper, which is then burnt as described.

The method has been developed quantitatively. A standard solution has been prepared containing the various elements in known quantities. Four different volumes of this solution are burnt on filter paper and the resulting spectra photographed on each plate on which the tissue spectra are recorded. The intensity of a given line in a tissue spectrum can then be matched with the corresponding line on one of the standard spectra, and the quantity of the element concerned can consequently be deduced.

Spectrographic analysis has hitherto only been applied to animal tissues in a few isolated investigations. Yet the method is obviously of great value, for a wide survey of tissue contents can be made with a rapidity impossible with chemical methods. We have commenced our investigations with annelids (analysed whole), molluscs (separate organs studied), and a few members of other phyla. Already a number of important new facts have emerged. The most salient are the following.

Iron and copper were present in all of the 146 different spectrograms made. The wide distribution of iron in protoplasm is already known, and its functional importance has been emphasised by Keilin's work on cytochrome (*Proc. Roy. Soc.*, B, **98,** 312; 1925). Copper has previously been found in numerous animal tissues and it has at least two functions, namely, as a component of the hæmocyanin molecule, and as an essential factor in hæmoglobin synthesis (Waddell, *J. Biol. Ch.*, **84,** 115; 1929, and others). When whole animals were used for our work, it is possible that elements which appeared in the spectra were in the gut or on the skin, and, of course, in the case of molluscs, copper exists in hæmocyanin. Nevertheless, the invariable presence of iron and copper strongly suggests that these are universal constituents of protoplasm.

Manganese was found in all nineteen species of polychætes studied. It was widely distributed in the molluscs, being present, for example, in all organs of land gastropods. It was found in numerous organs of marine gastropods, but was absent from *Haliotis*. The quantity in an organ varied with locality. Manganese is known to stimulate growth of rodents (McHargue, *Am. J. Physiol.*, **77,** 245; 1926. Bertrand and Nakamura, *C. R. Ac. Sci.*, **186,** 1480; 1928). When our survey has extended further, indications may be given of other functions. Harrison and Garrett (*Proc. Roy. Soc.*, B, **99,** 241; 1926) induced melanism in Lepidoptera by manganese feeding. We do not find this element particularly associated with melanin. The spectrum of the ink sac of *Sepia* shows none, and while manganese is strong in the black body-wall of *Arion ater* (0·008 per cent of dry weight), there is even more in the colourless common genital duct.

Nickel and cobalt were present in certain tissues. Cobalt was less common than nickel, and was usually associated with a relatively high concentration of the latter. But the foot of *Haliotis* has a high nickel content (0·004 per cent) without cobalt, while the liver of *Archidoris tuberculata* contains cobalt (0·003 per cent) with no nickel. There is thus a selective absorption.

Lead and silver occur spasmodically but not infrequently. The strongest silver was in the liver and kidney of *Pinna pectinata*. Individuals and individual organs absorb these metals selectively, for unknown reasons. One human subject had silver in all organs, while two others had none. Lead was present in different organs in each of the three men. This suggests an absence of functional significance.

Cadmium was found in the livers of all the eleven individuals of *Pecten maximus* examined. They came from three different localities. This seems to be the first record of cadmium in an animal.

Lithium turned out to be very widespread in animal tissues. Rubidium is less common. Cæsium was not found. This is remarkable, since rubidium and cæsium both enter frog's muscle from a perfusion fluid (Mitchell, Wilson, and Stanton: *J. Gen. Physiol.*, **4,** 141; 1921), and cæsium is present in sea water.

Strontium was present in 17 out of 19 species of polychætes and in 51 out of 67 tissues of molluscs. Previously it was known only from the skeleton of a radiolarian and from marine shells. Barium, on the other hand, was absent.

Calcium fluoride was found in one tissue only, namely, the body wall of *Archidoris tuberculata*.

A detailed account of the work will be published shortly.

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Experiments on Binaural Sensations.

AT intervals during the past five years I have been attempting a survey of the various experiments which have been taken as evidence that binaural sensations with musical notes of low pitch are due to the appreciation of phase differences produced at the ears. The work grew out of some experiments with sound waves which are recorded in the *Proceedings of the Physical Society* for August 1927.

Repetition of the various experiments has driven me to the conclusion that, with the forms of apparatus used, there have been possibilities of intensity changes and I do not think that these possible alterations have been sufficiently taken account of in the interpretation of some of the published results. It is very difficult to produce the binaural sensation of change of position of the sound 'image' under conditions where it can be shown that phase differences are set up without any accompanying alterations of intensity. Changes