

the blastopore marked *or* in figure 9 corresponds with the position of the permanent mouth, but before the widening of the permanent pharynx is formed it becomes exceedingly small, and I do not feel sure as to whether it closes up entirely as I previously maintained or whether it remains throughout (as Lereboullet believed), being at one period an exceedingly small aperture. In any case we cannot say that there is a direct conversion of the blastopore into the mouth, but rather a coincidence of the stomodæal invagination (invagination to form the Vorderdarm) with one end of the elongated blastopore. The middle third of the blastopore corresponds strangely enough with the position subsequently occupied by the foot.

The proctodæum or anal invagination does not occur till very late in *Limnæus*. The rectal peduncle is blind as in *Pisidium* and *Cyclas*; it must not be confounded with the Hinterdarm or Proctodæum, but is a part of the Urdarm or Archenteron.

NOTE ON MIHAKOWICS' NEW METHOD OF IMBEDDING. By
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IN a late number of the 'Archiv für Mikroskopische Anatomie' (II Bd., 3es Hft., p. 586) a new method of imbedding animal tissues for the purpose of preparing fine sections of them is described by Mihakowics, who used the method for the investigation of the vertebrate embryos. I have employed Mihakowics' method, with some very slight modifications, in the preparation of sections of decalcified corals, especially the *Stylasteridæ*, with great success. In all methods hitherto employed the imbedding substance has had to be removed by solutions from the interstices of the section of tissue before the section could be mounted and rendered transparent for examination. In the present method a substance is used which does not require removal, but is maintained *in situ* without diminishing the transparency of the object. The substance holds the delicate parts together, and maintains them in their relative positions in the section, whereas in methods where soap, &c., are used, the parts separated by section swim away most

provokingly all over the slide on the removal of the imbedding substance by benzine, oil of cloves, &c. The imbedding substance made use of by Mihakowics consists of a jelly composed of equal parts, by weight, of glycerine and gelatine. The glycerine is heated in a warm bath, and the gelatine dissolved in it. An extremely tenacious jelly is the result. I found this jelly too tough for use with corals, and I added a larger proportion of glycerine. The tenacity of the jelly can be modified to any extent according to the nature of the substance to be cut, just as the consistence of the oil and wax mixtures is varied to suit the tissue to be imbedded; but it must be remembered that the less tenacious the jelly, the more it will shrink when placed in absolute alcohol. Mihakowics stained his hardened embryos, then placed them in absolute alcohol, and, just before putting them in the imbedding substance, placed them for a minute in water in order to avoid great shrinking, which otherwise occurs. I hardened my corals in chromic acid, absolute alcohol, or osmic acid, decalcified them in weak hydrochloric acid, and then soaked them in glycerine, previously staining those hardened in absolute alcohol. The corals were transferred directly from the glycerine to the warm jelly. I found that the jelly more thoroughly penetrated the spongy tissues when they were thus previously soaked in glycerine. The imbedding jelly is kept just fluid over a water bath, and the small masses of tissue are macerated in it for one or two hours until all their interstices are thoroughly permeated by the warm jelly. In the case of corals, which, by the removal of the supporting calcareous skeleton become excessively spongy, it would be well, no doubt, to place the vessel in use under an air pump for a short time. There was, unfortunately, no air pump available on board H.M.S. Challenger. When the tissues have been well soaked in the jelly they are transferred, together with a portion of jelly, to small cavities scooped out in small blocks of liver which has been hardened in ordinary alcohol, and are arranged in the proper position for cutting, and the cavities filled up with the jelly. When the jelly has set, which condition most rapidly ensues, the blocks of liver are placed in absolute alcohol, and allowed to remain there two or three days. The jelly becomes hard, white, and opaque, and the liver hardens, and shrinking around it holds it firmly. The liver and hardened jelly are now cut into sections in the ordinary way with a razor, wetted with absolute alcohol, and the sections are treated with glycerine upon the slides. The opaque jelly becomes in a few minutes perfectly transparent, and is

almost invisible in the preparations. Some difficulty is experienced in transferring the tissue and warm jelly to the cavity in the liver before the jelly sets or becomes stringy. A teaspoon, previously heated in boiling water, is a most convenient instrument for the purpose. This method supplies a want which has long been felt. It is eminently adapted, for the preparation of sections of structures contain an abundant calcareous skeleton, and which are apt to collapse on the removal of this by acids, such as Echinoderms, corals, &c., but no doubt it will yield equally good results in such structures as Corti's organ, insects' eyes, &c., where it is desirable to retain *in situ* in a section a number of elements which have no organic connection or a very slight one with one another.