Studies in Spicule Formation.

V.—The Scleroblastic Development of the Spicules in Ophiuroidea and Echinoidea, and in the Genera Antedon and Synapta.

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With Plates 3 and 4.

Introductory.

The greater part of the material utilised in this inquiry was obtained from the Marine Biological Laboratory at Plymouth, and consisted of two common examples of the Ophiuroidea, viz. the small Amphiura elegans (Amphiuridæ) and the much larger Ophiothrix fragilis (Ophiothricidæ), and one of the Echinoidea—Echinus esculentus. Recently-metamorphosed specimens of this latter were mostly obtained from the artificially-reared plutei referred to in Study III (**13**), and were about a score in number; however, one or two slightly larger specimens were also secured from dredgings. Most of my results have been obtained from observations on the viviparous Amphiura elegans. In this Ophiurid the bursæ, as is well known, function as brood-chambers, and, in consequence, young animals are very easy to obtain in every stage of development. The methods of preparation adopted were as follows:—A hundred or more living Amphiuras being obtained, the disc of each animal was cracked, so as to permit the free access of the fixing, staining, and preserving reagents to the young animals contained within the bursæ, and these were then fixed with 1 per cent. osmic acid, stained with picro-carmine, and preserved in 90 per cent. alcohol in the manner already described in previous studies. Some of my best slides, however, have been obtained by merely fixing the live Amphiuras in absolute alcohol, subsequently staining for a fortnight or more in a saturated solution of safranin in absolute alcohol and washing out in absolute alcohol for a month or so after (if the alcohol be warmed less time will suffice). In many cases I also employed lichtgrün as a plasma stain, but, if employed, the solution in absolute alcohol must either be very weak, or, if a saturated solution, the Amphiuras must only be immersed in it for a minute or so, otherwise the tissues become opaque. Lichtgrün, when successfully employed, undoubtedly gives the best results; it is, however, quite possible to work without it, though in this case it is more difficult to be quite certain on occasion as to whether a particular cell belongs to a particular spicule or not. When the Amphiuras have been stained the discs are opened and the young ones extracted; these are then transferred to xylol, and finally mounted in balsam. In general only the youngest Amphiuras (about 0·5 mm. in diameter; see fig. 1) yield satisfactory results, though now and again it is possible to observe young spicules in the arms of the older Amphiuras. It is surprising how very few really satisfactory young Amphiuras are obtainable from numerous parents: from at least five or six hundred adults I have only managed to secure about a score of young ones showing the origin of spicules in an unmistakable manner.

The above-described methods of preparation were also employed in the examination of the specimens of Ophiothrix and Echinus.

My Antedon material consisted of very young specimens which, after having their discs opened, were prepared by the osmic and picro-carmine method. The imperforate thin plate spicules (which do not seem to have been previously described) are lodged in the hypostroma of the integument, and in consequence the portions of integument to be mounted must, when freed from the viscera and musculature, be placed inside upwards on the slide.

My Synapta material, consisting of S. hispida and S. digitata, was obtained from Naples, and I believe was simply fixed and preserved in alcohol. I stained portions of the body-wall by the safranin and lichtgrün method and obtained good results.

In many cases, especially of the older spicules of Amphiura, it is difficult to decide upon the exact number of cells in connection with a spicule, and such must be simply passed over. The figures provided in the accompanying plate were all drawn from spicules about which there existed no doubt whatever as to the number of scleroblasts attached, and these were only obtainable by careful and persistent searching.

The Origin of the Spicules in Amphiura elegans.

In Cucumariidæ (**13**) the plate-spicule originates as an elongated granule (thick-set needle) with its length disposed at right angles to the line joining the masses of the two scleroblasts usually concerned in its deposition; in some cases, however, four scleroblasts are concerned in the deposition, two being situated on each side of the needle. In Amphiura elegans, on the other hand, the plate-spicule originates in the same manner as the small superficial spicules of the Cucumariidæ do, viz. as an approximately spherical granule contained within a single cell (fig. 2). This difference of origin between the holothurian and the ophiuroid large plate-spicules can be correlated with other more general differences affecting the manner of lime secretion as it occurs in the two groups, and these I shall shortly indicate. For the present it suffices to point out that the needle and the granule correspond in form in both cases with the general space disposition of the scleroblasts concerned in their individual formation: the elongated needle is deposited by a binucleated mass of scleroplasm with maximum and minimum diameters in the plane of the body-wall, and the spherical granule by a scleroblast whose corresponding diameters are all approximately equal. The granule in A. elegans next assumes a flat three-cornered shape whilst still contained by the single scleroblast, and the three corners of the triangle thus formed then elongate to form a young triradiate spicule (fig. 3). Shortly after this stage is reached the nucleus of the scleroblast divides and two scleroblasts in consequence appear in connection with the young triradiate (fig. 4). Approaching nuclear division in the scleroblast is always denoted by large size and faint coloration by picro-carmine (fig. 5*a*, *e.g.*); as is well known picro-carmine is a stain which never renders the details of karyokinesis visible. The three arms of the spicule next show signs of bifurcation at the extremities (figs. 5, 6), and, indeed, the whole of the further development of the spicule (when this is not situated at the extreme edges or ends of the arms, in which position certain parts elongate greatly in a distal direction) consists, as in Cucumariidæ, of a series of bifurcations resulting in a more or less circular perforated plate (fig. 7), but it is noticeable that the attached scleroblasts differ from those of Cucumariidæ in their much greater number. The fact that abnormally-large nuclei are so often met with in these scleroblasts renders it exceedingly probable that they are all (thirty or forty in the case of the larger plates) derived from the original mother-scleroblast, although it is evidently impossible to make a decisive statement to that effect. This repeated division of the mother-scleroblast might, indeed, be attributed to the necessity for the proximity of nuclear substance to the constantly-increasing area of deposition (the well-known experiments of Verworn on Polystomella proving enucleated portions of protoplasm to be incapable of secreting a shell pointing the argument) were it not that no such subdivision occurs in the case of other spicules of equal size

(Study IV). Why in Ophiuroids (and all other echinoderms save holothurians) there should thus exist this definite relation between the number of attached scleroblasts and the size of the spicule, and not in holothurians I am quite unable to say. (As a remarkable illustration of this fact compare the stool spicules of Thyone [Study IV, fig. 55] with the young spines of Ophiothrix [fig. 13]).

Not all of the spicules are derived from an original triradiate structure; occasionally the spherical granule elongates to form a rod which bifurcates at its extremities, as in Cucumariidæ; occasionally also the young spicule assumes a more or less irregular form, and in Ophiothrix I have observed four- and six-rayed young spicules (figs. 10 *b* and *c*), but all these are very rare. The triradiate form of the young spicule is by far the most usual. It must also be mentioned that the largest of the perforated plates figured is small when compared with most of the spicules present in the young embryos of fig. 1, and of course the plates of the adult Amphiura are still larger by comparison.

The scleroblasts in Amphiura are spherical cells which assume a subspherical form when attached to the spicule; the nucleus is relatively large, and if stained with picrocarmine shows a distinct nucleolus. The cytoplasm is faintly granular. Nuclei vary in size to some extent even in the same individual, and, as already stated, become very large previous to division. In specimens fixed with absolute alcohol the nuclei appear somewhat contracted.

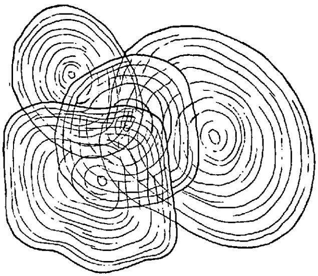
THE SPICULES IN OPHIOTHRIX FRAGILIS AND ECHINUS ESCULENTUS.

My material for ascertaining the mode of spicule formation in these two genera being very limited I have merely attempted to confirm the supposition that the process is here identical with that in Amphiura elegans, and this I have had no difficulty in doing. My Ophiothrix material consisted of several young specimens with discs about 1 mm. in diameter. Most of the plates in the young Ophiothrix are extremely thick, and with comparatively few and small perforations (fig. 11); however, the ordinary thinner plates are also present. The spicule originates in the same manner as that already described (figs. 8—10). The young forms represented by figs. 10 *b* and *c* are, as already mentioned, unusual. Fig. 10 *c* indeed reminds one as regards shape of a young stage in the formation of an auricularian wheel. Fig. 13 represents a young spine which develops in much the same way as the stool of Thyone, save of course that the basal plate originates as a granule in one cell, and that the number of scleroblasts concerned in its deposition is much greater. It is quite possible that not all the cells represented in this figure are scleroblasts, some possibly belonging to the surrounding membrane, and it is clearly impossible to distinguish between them; it is quite safe to say, however, that most of them are scleroblasts.

In Echinus esculentus (and miliaris) also the development of the spicule follows on the same lines (figs. 14—18).

The Plate-spicules in Antedon bifida.

The majority of the spicules situated in the region of the disc of Antedon lie towards the inner side of the soft integument, and are of two kinds—the imperforate thin "glasplättchen" of comparatively wide diameter and concentrically marked (see accompanying text-figure), and the ordinary perforated plates, which, however, often assume a very irregular shape, and occasionally become mere branching structures. All transitions (fig. 21) are to be found between the "glasplättchen," which are very unechinoderm in appearance, and the ordinary perforated plates; the former, however, are much the more common, and exist in great numbers. Since the development of the perforate plates and branching spicules is quite normal, i.e. the same as that already described for Amphiura, Ophiothrix, and Echinus, I shall merely describe the simple development of the imperforate spicules. These plates each originate as a spherical granule contained within a single cell (fig. 19), and this granule gradually becomes larger (fig. 20), and early assumes the plate-like form (fig. 22). There is thus no rod or triradiate stage—so common in the development of echinoderm spicules—in the growth of these plates, and the plate is never of the perforate type. The nucleus of the original mother-scleroblast divides at an early stage of growth of the spicule and the number of nuclei present at different stages of growth (a



Full-sized imperforate plates of Antedon bifida × cir. 270 diameters. These plates are very thin, easily fractured, show concentric lines (presumably of growth), and are to be found in large numbers in the hypostroma of the integument.

score or more on fully-developed plates) is strictly proportional to the size attained by the spicule. These large thin squamose spicules of Antedon do not seem to be generally known, although they exist in great numbers. They are very easily decalcified in virtue of their extreme thinness, and, as I have already mentioned, are very unlike echinoderm spicules (so much so that I at first mistook them for artefacts). As Dr. Bather has very kindly pointed out to me, similar, but much thicker, imperforate plates (with no concentric markings) have been described, among others, by Ludwig in Holothurians (**4**), Ophiuroids (**5**) (also Mortenson [**8**]), and Asteroids (Astropectinidæ) (**6**).

I may also mention that the dark granules, so conspicuous in the scleroblasts of many Cucumariidæ, are also present in the scleroblasts of Antedon; indeed, they are usually dark without any additional staining with lichtgrün.

THE SMALL PLATE-SPICULES OF SYNAPTA HISPIDA AND S. DIGITATA.

In addition to the plate-and-anchor spicules of S. inhaerens I have examined the similar (though easily distinguishable) spicules of S. hispida and S. digitata, and, as might have been expected, I find that the disposition of the scleroplasm concerned in their formation is essentially the same as that which I described in Study IV. Through lack of material I have not as yet been able to ascertain the disposition of the scleroplasm in the early stages of development, though I hope to do so shortly.

Besides these conspicuous plate-and-anchor spicules there exist in S. hispida and S. digitata (though not in S. inhaerens) small elongate plate-spicules, containing in the former species a single perforation in their centre, and the development of these perforate plates of S. hispida is remarkable. I may mention that these small plates are particularly numerous in the region of the muscle-bands, though they are also to be found in the intervening spaces.

The plate in both species arises quite normally as a more or less spherical granule in the centre of a single scleroblast (fig. 23), and, as in Antedon, this gradually becomes elongated (fig. 24) and plate-like, but, differing from Antedon, the nucleus of the scleroblast remains single throughout the entire development. In S. digitata these plate-spicules cease growth at the stage depicted in fig. 30, which, it will be noticed, is identical with the stage of development represented by fig. 24 of the plate-spicule of S. hispida. In this latter species the plate elongates considerably, and expands laterally to some extent except in the vicinity of the nucleus, the result being that the nucleus becomes lodged in a depression to one side of the plate (fig. 25). The further development of the spicule consists of the enclosure of the nucleus by the extension of the calcite, as shown in figs. 26—28. Two arms of calcareous matter extend round the nucleus on either side, meet, and finally fuse, and the adult plate, in consequence, contains a central perforation, in which the nucleus is imprisoned (fig. 28). Occasionally, when the perforation is larger than usual, a secondary ingrowth of calcite occurs (fig. 28*b*); occasionally also the two arms of calcite first overlap each other instead of fusing immediately (figs. 27*a* and 27*b*), and sometimes, but rarely, secondary pairs of arms are formed, which tend to emulate the first pair in their direction of growth (fig. 28*a*). This last feature (as also that represented by fig. 29) proves, however much it may appear to the contrary, that the presence of the nucleus is not the only stimulus giving rise to the peculiar mode of extension of the calcite just described. In fact, here, as in the cases described in the foregoing parts of this paper and elsewhere, the nucleus, with its associated mass of cytoplasm, probably has very little to do with the direction of growth of the calcite—with the form of the spicule—and must largely be discounted as a factor in the production of spicular forms.

THEORETICAL CONSIDERATIONS AND PREVIOUS WORK.

From the results described above, and from the figures of young spicules in the various classes of echinoderms provided by Agassiz (**1**), Ludwig (**7**), Seeliger (**9**), Fewkes (**2**, **3**), Théel (**11**, **12**), and many others, we may assume what has, indeed, been already implied, viz. that in Ophiuroidea, Asteroidea, Echinoidea, and Crinoidea, the typical mode of scleroblastic development of the spicules is that described above for Amphiura elegans, i. e. the spicule originates as a triradiate structure contained within a single cell. From the figures of these and other authors, on the other hand, we may also assume that the typical mode of development of the plates of Holothuroidea is that described by me for the Cucumariidæ (Study IV), viz. the origin of the elongated calcareous needle between two or four cells, its growth to form a rod, the bifurcation of the extremities of this rod, and so on. Up to the present I only know of one exception to this rule, Semon (**10**) describing and figuring most distinctly the triradiate mode of origin of certain spicules in the holothurian Chiridota venusta. But this, and possibly a few other exceptions, do not invalidate the general rule, and, as before mentioned, this difference of origin between most echinoderm spicules and the spicules of holothurians can be correlated with a general difference which exists between the modes of skeleton formation in the two groups, i.e. this rule can be justified by a reason for its existence. The quantity of lime respectively secreted by most echinoderms and by holothurians differs greatly—in the former group the stroma is packed with a calcareous stereom, whereas in most individuals of the latter the skeleton is only represented by isolated spicules—and correlated with this difference is (*a*) the fact that in the former group every scleroblast gives rise to a spicule, whereas in the latter at least two scleroblasts have to co-operate for the same purpose, and (*b*) the equally cogent fact that in most echinoderms scleroblasts multiply very rapidly (shown by the number of scleroblasts per spicule), whereas in holothurians they multiply very slowly. In other words, the difference in the origin of the spicule in the two groups is correlated with the amount of the skeleton present—with the skeleton-producing capacity.

Previous work on the subject of the present paper, so far as I have been able to discover, has been very small in amount. In fact, the only paper that I know of describing the origin of the spherical granule and the young triradiate in a single scleroblast is that of Semon (**10**) on the holothurian Chiridota just referred to. Semon also figures very distinctly the young triradiate with two scleroblasts (similar to fig. 4). At the same time Semon represents the triangleshaped spicule as a tetrahedron, and, curiously enough, represents this tetrahedron as forming the distinct centre or basis of the older spicules, secondary calcareous matter, so to speak, prolonging the solid angles of the tetrahedron. As I have stated in Study III, I believe this tetrahedron structure to be quite imaginary, and I certainly cannot credit without more evidence its persistence as the visible basis of older spicules.

Fewkes (2), describing the metamorphosis of Echinarachnius parma, says that "the first limestone formation which was observed is a trifid spicule in the wall of the body of the growing sea-urchin. In its very first form this trifid spicule is spherical in contour. Later it assumes a trifid shape, and seems to be enclosed in a transparent sac, the outer wall of which is believed to be formed of epiblast, the calcareous body being formed possibly in mesoblast"! Fewkes also adds that he does not know whether these trifid bodies develop into the plates or not.

Théel, in his papers on Echinus miliaris (12) and Echinocyamus pusillus (11), quite correctly describes the development of the spicules, and also states his opinion that "they first originate from cells which have wandered in between the tissues," but he gives no details of the scleroblastic development.

Ludwig, Fewkes, and others provide numerous figures showing the young triradiate spicules and older stages, but from not employing suitable staining reagents they entirely overlooked the scleroblasts in connection with the young spicules.

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EXPLANATION OF PLATES 3 and 4,

Illustrating Mr. W. Woodland's "Studies in Spicule Formation" (V).

Fig. 1 drawn natural size; Figs. 2, 3, 8, 9, 14, 15, and 23 × 1280 diameters; all other figures × 640 diameters. All figures (save Fig. 1) drawn with the camera lucida.

PLATE 3.

Development of the plate spicules of Amphiura elegans (Figs. 1—7), Ophiothrix fragilis (Figs. 8—13), and Echinus esculentus (Figs. 14—18).

Fig. 1.—Young Amphiura in which the spicules were observed. (Several specimens of Amphiura were considerably smaller than those here depicted.)

FIG. 2.—The origin of the plate-spicule in Amphiura elegans as a spherical granule contained within a single scleroblast.

FIG. 3.—The triangular and subsequently triradiate forms assumed by the initial granule within the scleroblast.

FIG. 4.—Young (usually) trifid spicules with two scleroblasts attached (derived from division of the mother scleroblast).

FIG. 5.—Older stage of the triradiate. In *a* one nucleus is dividing; in *b* one nucleus has divided, three scleroblasts being present.

FIG. 6.—The bifurcation of the arms of the triradiate. The number of scleroblasts has increased, and nuclei are seen to be about to divide.

FIG. 7.—Older spicules with numerous scleroblasts.

FIGS. 8—12 represent stages in the formation of the usually thick plates (with small perforations) of Ophiothrix fragilis. In Fig. 10, *b* and *c* represent young stages of unusual shape. Fig. 12 is a young spicule of the thin plate variety.

FIG. 13 represents one of the lateral trifid spines of O. fragilis. The numerous scleroblasts in connection with the spicule is striking when compared with the two scleroblasts associated with the somewhat similar "stool" spicule of Thyone (Study IV, pl. 34).

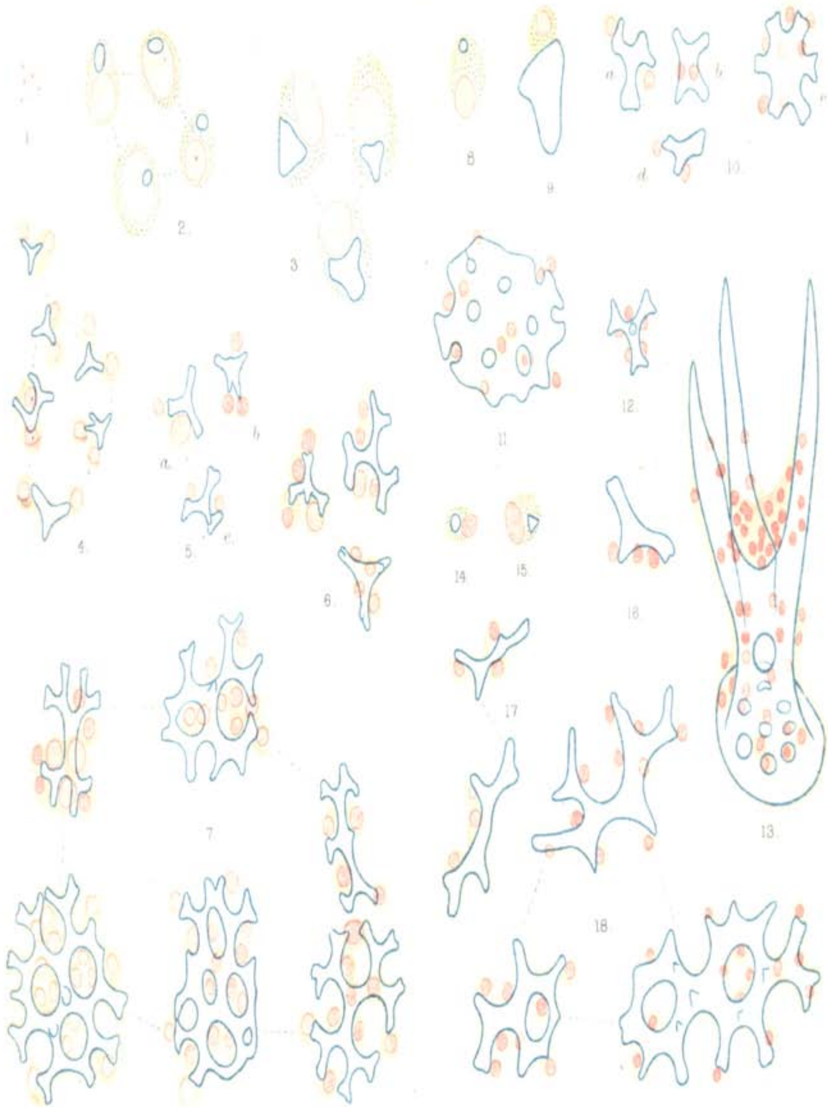
FIGS. 14—18 represent young stages in the formation of the plates in Echinus esculentus similar to those above.

PLATE 4.

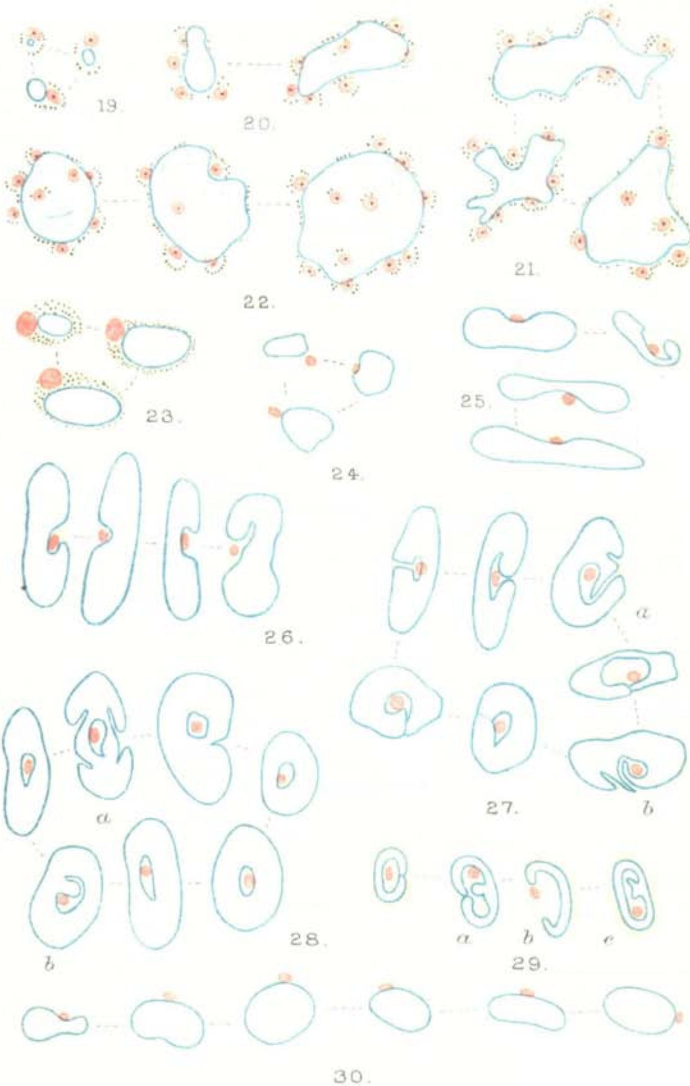
Development of the imperforate plate-spicules of Antedon bifida (Figs. 19—22), and of the plate-spicules of Synapta hispida (Figs. 23—29), and S. digitata (Fig. 30).

FIGS. 19—22.—The development of the imperforate plate-spicule of Antedon bifida. Fig. 21 represents spicules which show some affinity in their form to the ordinary perforate plate-spicules of echinoderms. The size attained by the adult thin perforate plate may be realised by comparing the present figures of young plates (× 640) with the text-figure showing adult plates (× 270).

FIGS. 23—30 represent the development of the curious plate-spicules o Synapta hispida and S. digitata, which are very small in comparison with the plate-and-anchor spicules. Fig. 24, representing a young stage in the development of the plates of S. hispida, shows forms very similar to those of Fig. 30, which represents adult spicules of S. digitata. Figs. 25—28 illustrate the enclosure of the nucleus within the central perforation. Fig. 29 shows plates in which the central aperture is considerably larger than the average.



SPICULES OF ECHINODERMATA.



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