Notes on Shell-Depositions in Oysters.

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With a

Note on the Chemical Composition of "Chalky" Deposits in Shells of *O. edulis.*

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With 3 Figures in the Text.

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INTRODUCTION.

The observations recorded in the following pages are regarded as preliminary studies—arising out of work on the bionomics of oysters, especially *O. edulis*—in the physiology of shell-deposition in these bivalves, and, it is hoped, they will be useful in the future in definite investigations designed to elucidate the bio-chemical factors and general conditions controlling shell-deposition.

The Distribution of Chalky Deposits on the Internal Face of the Shell of *O. edulis*.

During the examination of many thousands of shells of *O. edulis* the occurrence of chalk-like areas in a definite region on the internal face of the shell was observed to be almost constant. In these so-called chalky areas the crystalline nacreous layer of shell is replaced by material laid down in an apparently amorphous soft white mass, which is chalky in appearance and consistency. It was noticed that these deposits occurred mainly in the region of the exhalent chamber, but also in a position at the edge of the shell roughly opposite the palps and mouth. In order to obtain a definite expression of the situation of these areas, the internal faces of both valves in samples of shells were divided into arbitrary areas numbered 1 to 14 and lettered A to D, as shown in Fig. 1.

Samples of 100 shells from Thornfleet, West Mersea, and 100 from Brown Rose Bar, Fal Estuary, dredged respectively on October 7 and October 21, 1926, were examined and the distribution of the chalky deposits noted in each of the areas mentioned above, and in each shell.

Any area which contained either a large or a small deposit was recorded as showing a deposit.

The results of this analysis of the shells are given in Table I, p. 938, and are plotted to give the graphs in Fig. 2, p. 939. In Fig. 1, which shows the area-division of the shell, the areas numbered 1 to 9 fall in that part of the shell adjacent to the exhalent chamber, which is divided from the inhalent chamber—in the presumed extended condition of the gills—along a line represented approximately by the thick discontinuous curved line. The area divisions 10 to 16 bound the inhalent chamber; while the part of the shell in contact with the visceral mass of the oyster is divided into areas D, C, B, and in part A; but A is a meeting ground of visceral mass and both chambers, as also to a less extent is B. It was found that the deposits in area 1 to 9 were continuous whatever their extent, but those in 13, 14, and 15 were generally discontinuous and often occurred in small spots.

A glance at the graphs given in Fig. 2 shows that the chalky deposits are distributed in quite definite areas as follows:—

- In a high proportion in 2 to 9 in the region of the exhalent chamber (left valve).
- In a fair proportion in 7 to 9 in the region of the exhalent chamber (right valve).
- In a fair proportion in 13 to 16 in the region of the inhalent chamber (left valve).
- In a small proportion in 13, 14, and 16 in the region of the inhalent chamber (right valve).

But very rarely present in 10, 11, 12, and A, B, C, D, in both valves.

In the Mersea sample chalkiness is more general in occurrence but less in extent than in the Fal sample, a fact which is connected with the greater average age of the former sample, and variation due to different local physical conditions.

In both samples, however, the maximal occurrences of deposits fall in area 7 in both valves, and there is no doubt that the deposition of chalky shell-material begins in this locality, namely, about the middle of the exhalent chamber. The deposits in the right valve are less frequent and less extensive than those in the left, as is shown by the blackened areas—indicating deposits—in Fig. 1, below. Areas which had deposits mainly in 50 per cent or more of the shells (including also, however,

FIG. 1.

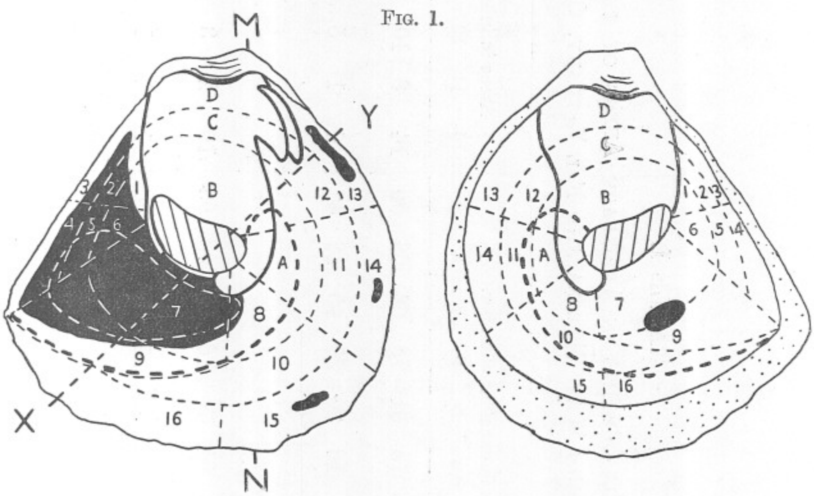

FIG. 1.—Diagrams of the right and left valves of the shell of *O. edulis*, showing
(1) the sub-division of the internal faces of the valves into arbitrary areas (separated by thin broken lines), and
(2) the mean distribution of the chalky shell-deposits on the internal faces of the valves in 100 shells from West Mersea, and 100 shells from the Fal Estuary, October, 1926 (age about 4 to 7 years).

The black regions denote the extent of the chalky deposits in areas affected in about 50 per cent of the shells.

The concentric region denoted by the dotted white line within the large black mass denotes the *extent* of deposits in this locality in about 30 per cent of the shells.

Areas numbered 1 to 9 form mainly the shell-boundary of the exhalent chamber on the sides.

Areas numbered 10 to 16 form mainly the similar boundaries of the inhalent chamber areas. D and C are adjacent to the visceral mass; B and A are mainly adjacent to parts of the visceral mass, but also abut on portions of both mantle chambers. The thick broken curved line marks the approximate dividing-line between the inhalent and exhalent chambers.

The visceral mass is shown in outline by a thick continuous line and the adductor muscle impression by close-set parallel lines.

The dotted region in the right valve shows the extent of the periostracal portion of this valve.

X–Y is the plane of section of the upper section shown in Fig. 3, p. 941.

M–N is the plane of section of the lower section shown in Fig. 3.

TABLE I.

Percentage Frequencies of Chalky Shell-deposits in Arbitrary Areas of the Shell of *O. edulis* in Samples of 100 Individuals from West Mersea and Fal Estuary, October, 1926.

	Areas in exhalent chamber.									Areas in inhalent chamber.							Areas adjacent to visceral mass.			
West Mersea—	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	A	B	C	D
Left valve . .	46	53	64	78	81	78	82	66	59	14	8	10	56	58	54	38	5	6	3	5
Right valve . .	12	14	8	16	30	25	45	18	42	6	1	5	27	23	3	20	3	2	1	2
Fal Estuary—																				
Left valve . .	23	34	42	61	75	76	93	68	63	15	2	3	34	39	38	34	8	1	1	1
Right valve . .	9	14	5	8	18	21	56	29	40	1	0	1	12	16	0	8	0	0	0	0

area 1, right valve, and area 7, left valve, in the Mersea sample, and areas 1, 2, 3, 13, 14, and 15, all of left valve, in the Fal sample, all of which areas had rather less than 50 per cent of shells with deposits) are included in the black areas shown in Fig. 1, and the extent of the deposition in 30 per cent of the shells, whose deposit in the exhalent chamber was small, is shown by the concentric white line within the biggest black area.

Deposits of chalky shell-material rarely occur in areas 10, 11, 12, A, B, C, D ; but when such do occur in these regions they form extensions of the main deposits, and occasionally may extend to cover the whole of the shell except the part touching the visceral mass, and in still rarer cases may cover the whole of the shell. This latter condition has been

Fig 2.

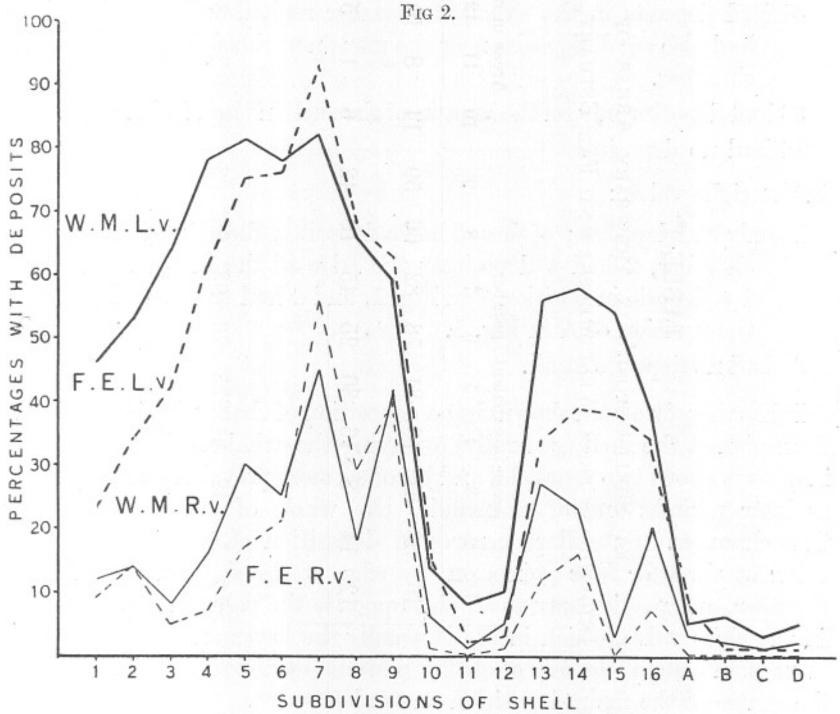

Fig. 2.—Graphs showing the percentage distribution of chalky deposits—of any extent—in arbitrary sub-divisions of the internal faces of the shell-valves in 100 *O. edulis* from West Mersea, and 100 individuals of the same species from the Fal Estuary (October, 1926).

W.M.L.V. The thick continuous-line graph denotes frequencies of chalky deposits in the left valve in the sample from West Mersea.

W.M.R.V. The thin continuous-line graph denotes similar right valve deposits in the West Mersea sample.

F.E.L.V. The thick broken-line graph denotes frequencies of chalky deposits in the left valve in the sample from the Fal Estuary.

F.E.R.V. The thin broken-line graph denotes similar left valve deposits in the sample from the Fal Estuary.

found to be unusually common in oysters dredged from 12 to 15 fathoms in the Parsons–St. Just Channels in the Fal Estuary.

In very young oysters—1 to 2 years old—chalky deposits occur fairly frequently and irregularly, but obviously in places where a thick layer of shell-material is required to fill up a space. In 2 to 3 year-old oysters shells with exposed deposits are rarer than in slightly older oysters from the same bed, and the deposits are less extensive, but the chalky areas are regular and show a concentration around the positions shown in Fig. 1 and especially in area 7.

In a sample of 91 left valves and 90 right valves, from mainly 2-year-old oysters dredged in July, 1925, on the South Shore at West Mersea, in the left valve,

41 had deposits in the exhalent chamber region, and a few of these had also small deposits near the margin of the shell in the inhalent chamber.

38 had deposits only on the margin of the shell in the inhalent chamber.

12 had no deposits.

In the right valve :

10 only had deposits ; of these 5 had a deposit at the edge of the exhalent chamber, 2 had a deposit at the edge of the inhalent chamber, 1 a deposit in position 7 in Fig. 1, and 2 had traces of deposits in the position of A in Fig. 1.

80 had no deposits at all.

It has therefore been shown that a deposition of chalky shell-material on the region of the shell (generally) overlying the exhalent chamber begins in oysters about two years old, and becomes more prevalent and extensive at later periods, and often includes the whole of the shell covering that chamber. Subsidiary centres of deposition of the same material occur at three or four points on the edge of the shell in the inhalent chamber, and particularly at a point opposite the palps in the left valve. In the right valve, which in life is usually the lower one, there is rarely more than a slight deposit of chalky material on the shell about opposite the centre of the main deposition on the left valve.

The chalky areas described above are visible on the internal face of the shell, and are sometimes powdery on the surface and in some cases covered with a nacreous deposit of shell-material. Sections of the shell show that a chalky deposit is not necessarily exposed on the face of the shell throughout life. A section of a shell with a large chalky area—taken in the plane X–Y, shown in Fig. 1—is drawn in Fig. 3, Y–X. In this section it is clear that successive chalky deposits—shown in black—occur alternating with varying thicknesses of nacreous deposits, and that recent deposits are nearer the edge of the shell than older ones, which, however, would have had a similar relation to the edge of the shell in the younger individual. There may, therefore, during the life of an oyster, be a succession of chalky deposits, which occur in the same relative position on the shell with or without alternating deposits of a crystalline nature. It is hoped later to obtain chemical analyses of the chalky and crystalline material from the same shell, but the analysis of the chalky material as given on p. 953 shows that it has essentially the same composition as the nacreous material.

A Note on the Nature of the Chalky Deposit.

The chalky deposit is composed of very soft material, which easily powders when cut with a knife, and though apparently amorphous to

Fig. 3.

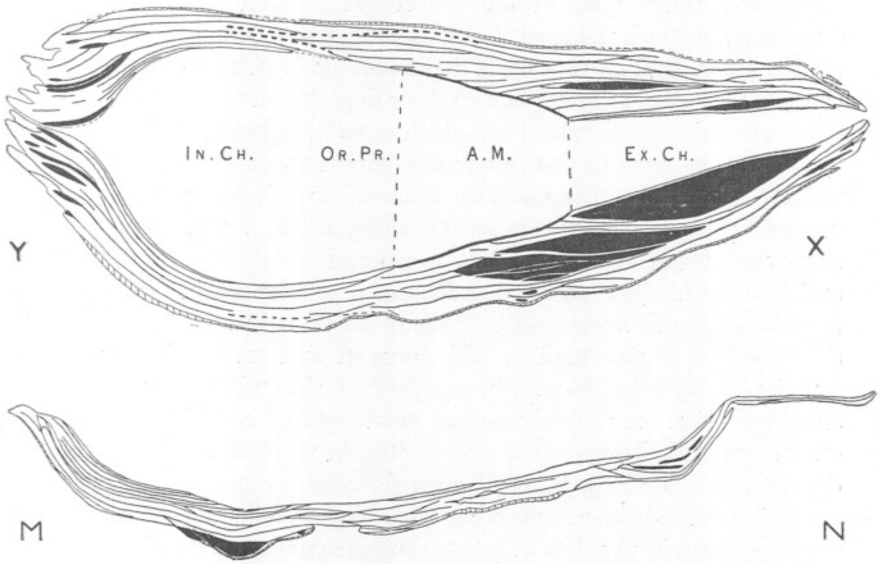

Fig. 3.—Sections of shells of *O. edulis*, with a polished surface, viewed as opaque objects.
Y–X. Sections of both valves of a shell from West Mersea, October, 1926, taken in the direction X–Y shown in Fig. 1. (Age unknown.)
N–M. Section of a left valve of a shell from the Fal Estuary taken in the plane M–N shown in Fig. 1. (Age estimated as certainly not more than five growth-years.)
Chalky deposits are shown in black.
Crystalline layers are shown only by their boundary lines.
Conchyolin deposits are shown as white areas, broken up by short close-set vertical lines, or, in Fig. 3, Y–X, in portions embedded in and overgrown by crystalline shell-material, by a thick black broken line.
In. Ch.=inhalent chamber in section.
Or. Pr.=region of oral process cut by the section.
A.M.=region of adductor muscle.
Ex. Ch.=exhalent chamber in section

the naked eye, appears to have a microcrystalline structure when examined through a petrological microscope.* Carpenter (1844) noticed these chalky deposits on oyster shells in his work on shell-structure, but he considered that such deposits did not form part of the proper structure of the shell, and states merely that they are composed of particles of carbonate of lime deposited in a chalky or concretionary state.

The crystalline structure of this chalky material is, however, peculiar, and will need to be investigated in a research on its mode of deposition. When microscopic fragments are examined with a high power an apparently organic structure—similar to but finer than that figured by Carpenter (l.c. Figs. 20–21, Plate 9) in shells of *Lima scabra*—can be seen. This structure consists of exceedingly fine branching and anastomosing rod-like bodies—determined by Carpenter in *Lima scabra* as organic tubes—apparently lying in lamellæ on and between which the calcium carbonate, of which the mass is largely composed (see a chemical analysis by Mr. H. O. Bull on p. 954), is deposited. It is an interesting fact that Orton (1923, Part 1) found in excretory deposits on the shells of oysters numerous microscopic platelets, which were interpreted as degenerating leucocytes, because of the occurrence within them of small branching rod-like filaments. These latter filaments are, however, very similar in size and general appearance to those found contributing to the structure of the chalky deposits mentioned above, and as Carpenter and Bowerbank both saw indications of a cellular origin of the similar branching organic structures in shells of *Lima scabra*, it is possible that shell-material of this kind is laid down in and on special leucocytes functioning as calcoblasts. In support of this view may be added an observation on the microscopic characters of a calcareous deposit on an oyster shell opposite a suppuration in the body. The calcareous deposit was exceedingly thin and covered numerous small cells or platelets up to 10μ in diameter containing branching rod-like structures similar to those found in platelets in brown spots (excretion on the shell covered with conchyolin only). At the edge of the calcareous layer was a network formed by chains of living cells (10μ in diameter in the nucleated portion), and in the calcifying portion a reticular organic (conchyolin ?) network which was obviously the basis for the beginning of the process of calcification, as slight calcareous deposits could be seen lining the network on each side. Within the calcified portion of the membrane solid concretions were deposited. Jameson (1912) figures similar concretionary deposits.

Thus there is good reason to infer the existence of a process of formation of shell-material, in which some type of leucocyte is intimately concerned, and the large granular type, which varies in number in different

* We are indebted to Mr. Palmer for assistance in this determination.

oysters, may be especially suspected. The total quantity of leucocytes in oysters is subject to very great variation, as Orton (1923) has previously pointed out, and may be said to be generally small in oysters with well-grown shells, and great in individuals with slow-growing shells (dumpy oysters), and relatively great in individuals which have fairly recently spawned as females.

THE DISTRIBUTION OF CHALKY DEPOSITS ON THE INTERNAL FACE OF THE SHELL OF *O. angulata*.

In the Portuguese oyster, *O. angulata*, deposition of chalky material on the internal face of the shell is much more prevalent and abundant than in *O. edulis*, and is also much more general. An analysis of the position of such deposits in this oyster has not been attempted because of their very general distribution, but it is probable that the region of the shell abutting on the main part of the visceral mass would be found to be less affected by the deposits than any other part. The flat (right) valve in this oyster has chalky deposits much more frequently than that in *O. edulis*. The general characters of these deposits in the Portuguese oyster are very similar to those in the very young *O. edulis*, that is, the deposits have always the appearance of filling up hollows, crevices, and other spaces. Indeed, they frequently seem to have been formed by the pouring out of a liquid matrix into a hollow with subsequent hardening. In the umbo of *O. angulata* there is normally in individuals of medium and advanced ages a deep recess. In a good proportion of cases, however, this recess is filled in with a plug of chalky shell-material which is smoothed off to run into the general contour of the adjacent shell.

Very deep shells of this species frequently have the bottom of the shell-cavity also filled in with a very thick similar deposit. In shells which have been bored—for examination of the internal organs—and have recovered after being put back in the sea, a heavy deposit of chalky material occurred in a few cases around the region of the boring. Such a deposit of chalky material never occurred in bored shells of *O. edulis*, although a much larger number of the latter were experimented upon.

In shells of *O. angulata*, therefore, chalky deposits are much more common and general than in *O. edulis*, and afford one more character of physiological difference between these two species.

ON THE FUNCTION AND CAUSES OF THE CHALKY DEPOSITS.

The cause of the formation of chalky shell-material in the place of the normal crystalline layers has not been demonstrated, but we believe that the explanation now offered will lead to its satisfactory demonstration by experiment in the future.

The formation of soft shell substance, the chalky depositions herein described in the shells of *O. edulis*, is discussed by Southern (1916). After explaining the formation of chambers by the deposition of layers of crystalline shell-material by the epithelium covering the visceral mass of oysters which have recently spawned—a view shared by Orton and Worsnop (1923)—Southern states that: "In order to accommodate the renewed increase in size of the body at the approach of the succeeding spawning* season, the *gonadial cavity* is again enlarged by the rapid deposition of soft shell substance in that part of the shell which *surrounds* the gonadial cavity. The alternation of hard and soft layers of shell substance is clearly shown in sections of the shell. In young oysters and in large (old) oysters without chambers, the shell is relatively thin, with very little soft shell substance."

It may be observed from this quotation that Southern is discussing mainly the function of the soft shell substance, the chalky deposit, in *chambered shells*, while the writers are discussing the function of the same material mainly in normal shells, but it is submitted that the explanation offered of the occurrence of these deposits in normal shells will hold also for abnormal chambered ones. The argument advanced by Southern can best be understood by reference to Fig. 3, Y–X, p. 941. The gonadial cavity referred to is the space occupied by the visceral mass and is well shown in Fig. 1, while the space (Or. Pr.) shown in Fig. 3, Y–X, to the left of the line joining the dorsal margins of the adductor muscle impression constitutes only part of that cavity. If a section similar to this had passed through the hinge, the whole of the space to the left of a similar line would belong to the gonadial cavity. Southern states that the volume of this gonadial cavity (in chambered shells, but normal shells are similar in this respect) "is enlarged by the rapid deposition of soft shell substance in that part of the shell which *surrounds* the gonadial cavity."

This statement is, however, demonstrably inaccurate. Any thickening of the shell outside the gonadial cavity will merely decrease the volume of the mantle cavities, as a glance at the figures will show. In order to increase the volume of the gonadial cavity the valves of the shell must be made to separate more widely (since absorption of shell-material need not be considered) on the dotted line in Fig. 3, Y–X, when the shell is occluded. This divergence of the shells is normally effected by growth of the shell substance at the periphery remote from the hinge. In this regard it has been found that a new rim of shell on the left valve can be formed at a very rapid rate at the beginning of the growing season,

* The word "spawning" has been used to replace "spatting" in Southern's quotation, for the sake of clearness, as spawning, and not spatting, is obviously meant. The italics used in this quotation are the writers'.

which precedes the breeding season. One cannot observe new shell growth on the right valve from external inspection, but it is not unreasonable to suppose that new growth on that valve will occur simultaneously with that on the left. If shell growth occurs in both valves, then—but only then—will the valves diverge and the gonadial space be increased.

A temporary increase in the general shell space could be attained by simple relaxation of the adductor muscle, and a fairly effective closure of the shell could be made even under these conditions, owing to the fact that the edge of the right valve is not calcified (see Fig. 1), but consists of a somewhat extensive and elastic rim of periostracum. In the fully occluded valves the calcareous rim of the right valve fits well inside the rim of the left one, and may leave upwards to $1\frac{1}{2}$ centimetres of the latter covered only by periostracum in a shell 8 centimetres in length.

It is clear, therefore, that an oyster can quickly increase the volume of the occluded shell in the growing season, or at any time temporarily by relaxing the adductor muscle, but in the latter case the shell would not be fully occluded. Although divergence of the valves may be desirable to give the visceral mass room to enlarge as the gonadial products ripen, or fattening or growth occurs, it is also possible for this mass to expand anteriorly and to a less extent posteriorly—or sideways in the view of the shell shown in Fig. 1—without any alteration in the disposition of the valves.

The function of the soft or chalky deposits in the shell of *O. edulis* is therefore not that of increasing the size of the gonadial cavity.

It is submitted that the general character of the depositions in *O. edulis* and *O. angulata* show that the function of these deposits is to fill in rapidly depressions under the mantle, or secreting epithelium, which depressions cannot be maintained in the physiological state of the oyster at that instant, or which can only be maintained with loss of efficiency in functioning.

The general occurrence of amorphous deposits filling in unwanted space, as in the umbo and in deep depressions in the shell in *O. angulata*, in crevices and to bridge over crevices in the very young *O. edulis*, and in deep grooves in all ages of *O edulis*, indicate the function of reducing mantle space at a rapid rate. Nothing is yet known of the rate at which chalky deposits are laid down or when, but the thickness of the deposits in very young oysters—and in old ones—proves that the rate of secretion is rapid in comparison with that of nacreous shell.

Although the function of the deposits noted above seems clear—and the absence of contact of mantle with shell may be regarded as the stimulus which causes such deposits—the occurrence of the large mass of chalky material in the shell over the exhalent chamber in *O. edulis* presented at first a baffling problem, but we now believe that the explanation of this deposit is the same as for others. The left valve in *O. edulis* is the convex one, and in life usually lies uppermost, and it is in this valve that the large chalky deposits occur ; in the right valve the deposit of the same material is smaller, and occupies a corresponding position, namely, round about the middle of the exhalent chamber. The part of the mantle which covers the chalky deposits consists of a large triangular thin sheet of tissue, which is capable of contraction and extension, and, no doubt, in the exercise of the normal functions of life—particularly in the evacuation of fæces—is constantly extended and contracted. It is suggested that during these movements the central portion of the mantle is restored to its position of close contact with the shell with difficulty, especially in shells in which the contour of the exhalent chamber is markedly concave. If this difficulty is a real one the mantle will tend to sag at a point opposite about the middle of the exhalent chamber, and may form a cavity at this point by a local exudation of liquid under the reduced (suction) pressure. Under these circumstances the conditions will be the same as those observed above, and the stimulus being the same, deposition of chalky shell-material begins and is continued until the contour of the shell is such as to permit the maximum of efficiency in the extension and contraction of the mantle. There are grounds for the view that a contour like that of the right valve, namely, very nearly flat, is the most suitable one, as the effect of the chalky depositions is to flatten out the contour, and some shells moderately flat in the region of the exhalent chamber do not develop chalky deposits at all.

When the chalky deposit has resulted in the attainment of an efficient contour, it ceases, and crystalline deposition should recur. If after nacreous material has been laid down over a chalky deposit there occur a general growth of shell at the periphery, the mantle will extend on to the new growth, and the conditions which stimulate the production of chalky shell-material may recur, moreover, as a shell is usually enlarged in a manner which continues an established mode of growth a recurrence of similar conditions in the spatial contour of the exhalent chamber is possible, and in this way may render necessary a fresh deposition of chalky shell-material.

The similar deposits at the edge of the shell in the left valve (areas 13 to 16 in Fig. 1, p. 937) which occur in hollows and crevices are capable of similar explanation to that given ; but these deposits often form a smooth ridge in this region of the shell, and may then be regarded as having consummated their function of producing a surface over which the mantle can be contracted and extended with a maximum of ease. An additional cause for the formation of these border deposits may also arise from the functions of the ciliated path on the mantle, particularly at the point where this path receives rejected food-masses from the palps.

There is therefore good ground for the view that all chalky deposits of shell-material are due, on the whole, to local unsuitabilities of the contour of the shell to the needs of the oyster, and that the deposits are made to adapt the shape of the shell to the changing needs. In the young oyster shell there frequently occur numbers of grooves and hollows, but these gradually become smoothed out during the course of life in the process of the formation of a shell which has everywhere a smooth and gradual contour.

On reading the preceding discussion on the function of chalky deposits of shell-material, Dr. Allen raised the problem: Why should *O. edulis* form such depositions when *Pecten varians*, which is present on the same beds in great abundance, does not form, or only very rarely shows, similar deposits? The answer to this question brings out a point of essential importance. *Pecten varians* settles in the post-larval stage with a fully formed shell, and merely attaches itself inside or on shells by means of byssus threads, consequently the kind of surface on which the post-larva settles exerts no influence towards modifying the growing Pecten shell, which accordingly develops normally in its specific shape. The corresponding facts with regard to *O. edulis* are quite different: the shelled larva settles on an object to which it cements itself at once by a deposition of shell-material. From this point of development for a considerable time—one to two years—onwards the shape of the object on which a young *O. edulis* has settled bears a very intimate relationship to and largely controls the shape of the young growing shell. Thus the shape of the young shell in *O. edulis* is variable, and is not subject to the same control by the organism as is the case in *P. varians*. Since the shape of the young shell is variable, and if a particular contour of shell in the adult is desirable for a maximum efficiency in functioning, it follows that modification of the contour of the growing shell by internal depositions will often be necessary to attain the desired end. In a similar way variations in shell-shape may be expected to result from moving oysters from one situation to another, for there can be no doubt that there is a general uniformity in shell-shape in a particular habitat, and that a variety of habitats may occur even within the boundaries of one set of beds. Too little is known at present, however, of the relation of shell-shape and other shell characters to the habitat (as Sir Ray Lankester has pointed out in letters) to render further discussion of the problem profitable. It may nevertheless be mentioned that in oysters, especially *O. edulis*, from muddy bottoms in shallow estuarine situations, there is a great tendency to a flat habit of growth with considerable puckering or fluting of the shell in a direction radial from the hinge, while in deeper water and some open water forms, as in Whitstable oysters, this puckering of the shell is greatly diminished. It is not improbable that these shell formations, which are in turn determined by the shape of the mantle, are controlled largely by the feeding habits imposed by the local conditions, and we know very little about those particular feeding habits of oysters which are concerned in this problem. It is indeed probable that the feeding habits of young oysters are different from those of the adult. All these considerations, however, point to the probability of a changing contour of the shell, with the resultant necessity for the adaptation of the old contour to the needs of the new and changed conditions.

At the beginning of the investigation the chalky deposits in the shell adjacent to the exhalent chamber and those in the region of the palps were thought to be connected with the high degree of acidity of the juices in the alimentary canal of the oyster recently shown by Yonge (1926). It is now, however, regarded as unlikely that the high degree of acidity of the liquid passing through the exhalent chamber can have more than a minor significance, if any, in the process of deposition of chalky shell-material. When the physico-chemical reactions governing the deposition of this kind of material are known, it will be possible to reconsider the effect of the acid medium in the exhalent chamber. The occurrence of a slightly acid medium under the mantle and adjacent to the shell may, however, be an important predisposing factor in the formation of chalky material by attracting leucocytes to the locality.

It is hoped that the preceding discussion on the occurrence and distribution of amorphous deposits will lead to an investigation of the biological and physico-chemical controlling factors.

On the Discontinuity and Frequency of Deposition of the Nacreous Layers of Shell-material in *O. edulis*.

The section of shells of *O. edulis*, shown in Fig. 3, p. 941, are of interest in demonstrating (1) the discontinuity of the nacreous layers of shell-material, which are indicated by the blank spaces and separated and delimited by the sub-parallel lines, and also in showing (2) that more than one such layer may be laid down in one year of growth

The sections shown in Fig. 3 were made for the purpose of obtaining information of the disposition of the amorphous deposits, which are shown in black masses, and the discontinuity found in the nacreous layers has not been fully investigated. The opportunity is taken here, however, to make a few preliminary remarks on the subject. The section in Fig. 3, Y–X, passes through the exhalent chamber region, the adductor muscle impression, a portion of the gonadial cavity containing the oral process, and the inhalent chamber region, in the plane X–Y, shown in Fig. 1, p. 937. The main feature of the section is that there are more numerous layers of nacreous material at the edge of the mantle cavity of the shell than in other parts, and there is evidence of the deposition of definite layers from the edge of the adductor muscle to the outer edge of the shell.

In a study of shell growth these observations will need to be followed up in order to understand the whole mode of shell formation. The sections in Fig. 3 were prepared by cutting the shell in two and grinding down and polishing the cut faces.

The section M–N is of a thin broody Falmouth shell, about 65 mm. in length and of an age of certainly not more than five summers' growth. The line of section in this case is through the hinge, alongside the anterior edge of the adductor muscle impression, and across the region of the inhalent chamber, that is, almost a median dorso-ventral section. The number of nacreous layers in the hinge region is twice the number of years of the estimated maximum age. There is therefore no doubt that more than one nacreous layer may be laid down in certain parts of an oyster shell in one growing season. It has already been noted (Orton, 1925) that two or more shoots of growth may occur at the edge of the shell in one season, and there is an indication in this section that internal layers of nacreous material are laid down in the hinge part of the shell at the same time as the shoots of shell at the edge.

In the year 1926 samples of Falmouth oysters were examined throughout almost the whole of the year, and distinct periods in spring and autumn were observed when new growth at the edge of the shell was general. New growth of shell occurred during the summer, but not in the same general way as in spring and autumn. It seems highly probable that these rhythmic growth periods are a normal feature in the life-history of the oyster, and are probably intimately related to the general physiological series of seasonal changes in the organism.

The probability that there occurs a minimum temperature below which calcareous material cannot be deposited in *O. edulis* has been previously discussed (l.c. *Nature*, 1925), and in 1926 some definite data on the problem were obtained.

In October, 1926, in the Fal Estuary growth of shell-material at the edge of the left valve occurred extensively on all grounds. On October 7th the general sea-temperature over the beds was 61° F., and fell during the month to a general level of 52° F. on October 28th. During November the temperature varied about 50° to 51° F., when only very occasional individuals were found with thin recent shell-shoots, although the October shoots were then still very obvious and hardening.

A similar general correlation between cessation of autumn shell growth and temperatures between about 60° and about 54° had previously been noticed without, however, definite general observations like those given above being possible. There is therefore in these observations support for the view that when sea-temperature falls to a point not less than

52° F. prismatic—and probably also nacreous—shell-material ceases to be laid down in *O. edulis*, and conversely when the temperature rises above some temperature level between 52° and 60° F., shell-deposition will begin again in the spring. Shell-deposition began in the Fal Estuary in 1926 at some time earlier than April 22nd, when new calcareous growth of shell had attained a median dorso-ventral length of a few to 9 mms. in 53 out of a total of 73 oysters with normal shells examined. Definite temperature observations were not being made in the Fal Estuary at that time, but there can be little doubt that the sea-temperature had risen to a level which would approximate closely to that at which growth ceased in the autumn. It is hoped, however, that it will be possible to follow and correlate the general occurrence of new shell growth and general temperature variations on these beds in the future.

On Deposits of Horny Material on the internal Face of Shells of *O. edulis*.

A very large proportion of the shells of *O. edulis* from the beds in the Fal Estuary have extensive depositions of horny material, which is regarded provisionally as conchyolin, on the internal faces of both valves, but mainly in the areas 6 to 12 (in the region of the inhalent chamber) in Fig. 1, p. 937. In a fair proportion of cases these deposits extend over the remainder of the region of the inhalent chamber and also over the shell in the locality of the exhalent chamber. Nacreous layers of shell-material may be laid down over previous conchyolin deposits (see Fig. 3, Y–X, p. 941), which remain visible for a time through the translucent nacreous layer or layers. It was found, however, that the conchyolin skins peeled off the shells in samples examined in late autumn, but samples examined during the summer months were not noticed to exhibit this peeling phenomenon, and as special attention was not directed at the time to the state of the conchyolin layers, seasonal observations will be required to confirm the observations.

It would seem, however, that the conchyolin deposition occurs mainly in the autumn towards the end of the shell-growing period. As conchyolin is the normal organic basis, and probably forms a matrix, for the deposition of nacreous shell-material, it would seem that at the period when conchyolin is deposited, the physico-chemical—and perhaps in some cases the general physiological—conditions are unsuitable for the completion of a phase in the formation of nacreous shell-material, which had been initiated but could not be completed, and as a result conchyolin alone is deposited. This view is in accordance with the fact that crystalline shell-material ceases to be laid down at the approach of winter—probably at some definite temperature epoch—and with the probability that a general deposition of conchyolin alone occurs at the approach of winter. Further observations are needed to obtain definite seasonal data, but in the meantime it is clear that conchyolin formation may occur over the whole extent of the mantle and is not necessarily confined to a small layer of cells at the edge of the mantle where the normal external layers of conchyolin are produced to form the periostracum.

Whereas in the Fal Estuary oysters a proportion of over 90 per cent of the shells may have extensive conchyolin deposits on the internal face of the shell, it was found that a sample of shells from West Mersea in October, 1926, had only slight conchyolin deposits in the same situation in about 30 per cent of the shells.

Conchyolin depositions of this kind are certainly more frequent in Fal Estuary oysters than in samples from most of the beds from the Thames Estuary, a fact which appears also to be consistent with the view advanced above on the cause of the deposits, for sea-temperature falls much more rapidly between 60° and 50° F. in the Thames Estuary than in the Fal Estuary, so that a longer time for conchyolin deposition will be available in the latter locality than in the former. The occurrence of small membranes of conchyolin covering excretory deposits on the shell has been dealt with in some detail elsewhere (Orton, 1923); there is no doubt that these latter membranes serve a different function from the extensive conchyolin skins mentioned above, but they also show that conchyolin may be produced by the general outer surface of the mantle.

A Note on Pigmentation in *O. edulis*.

An additional feature of interest in connexion with the shell has been observed in some individuals of *O. edulis* from the Fal Estuary. In a small percentage of thin-shelled individuals—but in no other kind of oyster—it was noticed towards the end of the breeding season in 1925, and again in a few cases at the end of 1926, that black pigment had been laid down on the left side in the epithelium covering the visceral mass. It seems probable that the pigment may have been produced as a reaction to the penetration of the thin shell by certain actinic rays, for black pigment is abundantly produced on the edges of the mantle in oysters which there is every reason to believe have been situated so as to receive light rays.

The pigmentation of the mantle in samples of dredged oysters shows a very wide range of variation, which is probably correlated with the variation in incident light. A similar phenomenon has been noticed in *Cucumaria saxicola* and *C. normani*, whose tentacles become black when exposed to light, and in *C. saxicola* the skin becomes generally dusky black after prolonged exposure to light.

If some form of radiant energy can penetrate thin shells of oysters *in situ* in the water and produce a response in such a remote part of the body as the visceral mass, the phenomenon is probably worth further investigation.

SUMMARY.

The observations recorded form preliminary studies which it is hoped will be useful in special investigations on the physiology of shell-depositions in oysters. The distribution of chalky shell-deposits on the internal faces of the valves in young and old *O. edulis* and *O. angulata* has been studied, and its average distribution determined in shells of *O. edulis* of medium age from West Mersea and the Fal Estuary.

The distribution of this material in the shells of young *O. edulis* and in *O. angulata* is such that there can be little doubt that the function of the chalky deposit is to fill in rapidly grooves, hollows, and other spaces, which are inimical to efficient functioning in the changing needs of the individual.

In *O. edulis* of medium age large deposits occur regularly in the shell adjacent to the exhalent chamber and smaller ones on the border of the shell in the region of the inhalent chamber. The hypothesis is advanced that these regular deposits can be explained in the same way as those more easily understood by assuming that in a high percentage of shells of medium age, the contour of the shell in the region of the exhalent chamber and other parts is such that rapid extension and contraction of the mantle cannot be effected; deposition of chalky shell-material modifies this contour of the shell with certainty in some cases, but hypothetically in others, so that the mantle can be extended and retracted with a maximum of efficiency.

Sections of shells of *O. edulis* show that more than one layer of nacreous shell-material may be laid down in one growing season, and general field observations indicate that the normal number of nacreous layers deposited may be two or more in certain parts of the shell each growing season.

Two main periods of shell growth, i.e. growth of shell at the periphery of the left valve, were observed in the Fal Estuary in 1926; one in spring and one in autumn.

Observations are given which indicate that crystalline calcareous depositions cease in *O. edulis* at a temperature not less than and probably slightly above 52° F.

Conchyolin depositions on the internal faces of both valves in the form of extensive thin sheets are found in a very high percentage of shells

of *O. edulis* in the Fal Estuary, and in small areas in a fair percentage of shells from West Mersea.

A theory is advanced to explain these conchyolin deposits. Conchyolin can therefore be produced and deposited by the whole of the mantle lining the shell in *O. edulis,* as well as by the specialised cells at the edge of the mantle.

Black pigmentation has been observed on the visceral mass of thin-shelled *O. edulis,* a phenomenon apparently due to actinic rays penetrating the thin shell.

REFERENCES.

1844. Carpenter, W. B. On the Microscopical Structure of Shells. Rep. British Assoc., pp. 3 and 14.

1923. Orton, J. H. Min. Agric. and Fish. Fisheries Investigations, Series II, Vol. 6, Pt. I, p. 61, and Pt. II, p. 65. London.

1916. Southern, R. Chambrage in Oysters. Dept. Agric. Techn. Instruct., Ireland. Journ., Vol. XVI, No. 4.

1923. Worsnop, E., and Orton, J. H. The Cause of Chambering in Oysters and other Lamellibranchs. Nature, Vol. CXI.

1926. Yonge, C. M. Structure and Physiology of the Organs of Feeding and Digestion in *Ostrea edulis.* Journ. Mar. Biol. Assoc., N.S., Vol. XIV, No. 2, p. 370.

1925. Orton, J. H. The Conditions for Calcareous Metabolism in Oysters and other Marine Animals. Nature, Vol. 116, p. 13.

1912. Jameson, H. L. Studies on Pearl-Oysters and Pearls. Proc. Zoo. Soc., London, p. 260.

A Note on the Chemical Composition of "Chalky" Deposits on the Shells of *O. edulis.*

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The "chalky" deposit consists of a white, impalpable powder. Microscopic examination showed that it was mainly exceedingly fine crystalline matter, and did not appear to be a precipitated substance. Its general composition is shown in the following table. The analysis was made on a mixed sample of the "chalky" deposit taken from four separate left valves of *O. edulis* from the Fal Estuary, October, 1926. The approximate composition* is as follows :—

	%
$CaCO_3$	78·5
Water and organic matter . . .	19·2
Undetermined, including MgO, Na_2O, P_2O_5, SiO_2, So_3, Cl, and S. . .	2·3
	100·0

It is hoped, at a later date, to make detailed comparative analyses of the "crystalline" and "chalky" deposits of shells from different localities.

* The (water+organic matter) represents the loss on gentle ignition at a temperature below that causing decomposition of the $CaCO_3$, and weighed to a constant weight. Lime was determined in the usual way by precipitation as oxalate, weighed as CaO, and the result calculated to $CaCO_3$.