ON THE POSSIBLE EFFECT OF THE ENVIRONMENT ON THE CYTOPLASMIC INCLUSIONS IN THE OOCYTES AND OOGONIA OF *DASYCHONE CINGULATA, SALMACIS BICOLOR*, AND *CLIBANARIUS OLIVACEUS*.

By M. K. Subramaniam, M.A., D.Sc.,
and
R. Gopala Aiyar, M.A., M.Sc.
(From the University Zoological Research Laboratory, Madras.)

Received February 3, 1936.

Introduction.

Studies on the cytoplasmic phenomena during oogenesis in the same animal by different authors (for example *Lumbricus* Harvey, 1925; Gatenby and Nath, 1926; *Lithobius* King, 1924; Nath, 1924; Weiner, 1925; and Koch, 1925) have brought to light a remarkable diversity of results. Even a reinvestigation of these phenomena in the same animal by the same author (*e.g.*, *Lumbricus* Harvey, 1925 and 1931; *Pheretima* Nath, 1930 and 1933), shows no correlation. Taking *Lumbricus* Harvey in 1925 showed that there is a fair amount of yolk in the egg and he gives tests for distinguishing yolk from fat (pp. 299 and 306, 1925). But in his restatement of the oogenesis of *Lumbricus* he observes that the material, which he designated as yolk for want of a better term is really a weak fat. These fat droplets blacken in osmic, but in osmicated ovaries brought up through alcohols and mounted in balsam leave empty vacuoles. These according to him are able to resist alcohol only up to 70% dissolving in 90%. Nile blue sulphate stains these spheres very pale blue; in Sudan III they are pale yellow and after Scharlach R deep scarlet. Since these spheres were found to dissolve in 90%, how he was able to see them in finished slides (1925) and decide that they are yolk, giving at the same time tests, baffles one.

In *Pheretima* according to Nath (1930) the Golgi vesicles contain fat, and as a result in Champy fixed ovaries mounted whole appear as black granules, but within a month or so they are decolourised. In Champy fixed sections these vesicles are decolourised on immersion in Xylol.

Nath (1933) in his recent contribution (basing his observations on staining with Sudan III and Scharlach R) mentions that nothing in the oogonia or oocytes of *Pheretima* was stained by Sudan III or Scharlach R. He therefore, concludes that what the Golgi are composed of and also contain is a highly unsaturated or little oxidized lipoid.

Harvey (1925 and 1931) and Nath (1930 and 1933) in an attempt to correlate their results with the observations of other workers (Gatenby and Nath, 1926; Weiner, 1925 and 1930; Foot and Strobell, 1900 and 1901; Foot, 1894 and 1896) suggest that these differences may be due to the idiosyncrasies of the technique. For example, Harvey (1931) comes to the conclusion that what Nath (1930) considers as Golgi bodies in *Pheretima* are not Golgi bodies at all but that they are droplets already of a fatty nature. The view expressed by Harvey would appear to be untenable as Nath definitely states that the Golgi bodies in *Pheretima* appear as solid blackened granules after Da Fano (p. 495, 1930) and that even in Bouin preparations these distorted Golgi elements occur (p. 497) as spherules resembling yolk droplets. Harvey (1925) himself shows in the table extracted from the *Vade-Mecum* that fat vacuoles are not fixed in Cajal and that they are washed away by Bouin. Even Nath's interpretation of the fat content of Golgi vacuoles would appear to be erroneous because it is well known that Golgi containing fat cannot be seen as granules after Da Fano or preserved after Bouin. Further Nath (1933) doubts the existence of fat droplets, as reported by Harvey, as these give a negative reaction to Nile blue sulphate, a positive one to Scharlach R and a weak one to Sudan III. Lorrain Smith (1907) found that Nile blue sulphate stains neutral fats red and fatty acids blue. Tennent, Gardiner and Smith (1931) demonstrate that while triolein taken separately is stained red and oleic acid blue by Nile blue sulphate, mixtures containing a very small amount of oleic acid are coloured blue by this stain. The fat in eggs is not found in the pure state and as its composition may vary it is difficult to accept Nath's criticism. That Scharlach R and Sudan III cannot be depended upon is a very well-known fact and hence Nath's observations cannot be regarded as conclusive. And further, it would be interesting to know the action of turpentine on bodies that stain with Sudan III and Scharlach R.

The authors of the present paper feel from the results obtained that the main cause for differences in observation depends more upon other factors than on technique.

It has been shown by one of us in a previous paper (Subramaniam, 1935*a*) that differences in the physico-chemical factors of the environment is followed by differences in the quantity and mode of behaviour of the various inclusions. In the present paper we hope to dilate on those observations.

Literature.

Since Orton's work (1922) on "Sea Temperature, Breeding and Distribution in Marine Animals" innumerable papers have appeared showing the effect of temperature on breeding. In *Calanus finmarchicus* Russel

(1928) has shown that the generations vary not only in size but also in physiology. Bogorov (1934) has further demonstrated that there is an alteration in biomass and size during the various seasons. Biomass and size are small in February to mid-April and both reach a maximum in May. From June to December biomass and size remain stable after a slight decrease and the values reach their lowest level in October. Bogorov mentions that there is a definite relation between size and physico-chemical conditions of the environment, especially temperature. The results of Bogorov and Bogorov and Preobrajensky (1934) indicate that the average biomass of *Calanus* in Barent's Sea (where it produces only one generation a year) is equal to the weight of three generations of *Calanus* produced in the English Channel. The difference in temperature between 'spring' and 'autumn-winter' generation in the English Channel is about 8°C. and the biomass of 'spring' *Calanus* is twice as large as the 'autumn-winter' ones. The differences in temperature between English Channel and Barent's Sea is about 10°C. and the average biomass of *Calanus* in Barent's Sea is twice the value of that in English Channel. The northern and southern parts of the Barent's Sea show a difference of 5°C. and this difference brings about a three-fold difference in biomass. Life of *Calanus* in Barent's Sea is longer than that in the English Channel.

The breeding periods of *Echinus esculentus* (Moore, 1934) vary even in localities separated only by a few miles and at different depths. According to Moore (1934) temperature is the important factor that causes such variations.

Finally the chemical analyses of Plankton by Brandt (1898) and Raben and Brandt (1919) indicate a variation in the quantities of fat, protein and water content during different seasons. Orr (1934) finds that in *Calanus finmarchicus* from Loch Fyne, the fat content generally follows the weight curve but that it is highest at the beginning of March and lowest in May. Wimpenny (1929) working on the North Sea zoo-plankton records that maximum fat content was found in August and minimum in October. Similarly the percentage of protein in the body varies also during the different seasons (Orr 1934, Marshall, Nicholls and Orr, 1934). Orr further states that in March when fat content was unusually high a low value was obtained for protein content. The protein values varied from 50 to 35% and the percentage also followed the weight curve in general (Marshall, Nicholls and Orr, 1934).

A seasonal variation in the amount of glycogen and total carbohydrate content was noticed by Stott (1931) in *Echinus esculentus*, and he supposes that glycogen is transformed in maturing gonads into carbohydrate food

reserved for the ripe sperms and eggs. The records of Stott also show an individual variation in the fatty acid content of the gonads.

Bogorov (1934), Bogorov and Preobrajensky (1934), Moore (1934), Marshall, Nicholls and Orr (1934), Orr (1934), and Stott (1931) pay attention only to temperature. Temperature is but one of the factors. Pantin (1931 *a* and *b*), Weil and Pantin (1931), Beadle (1931), Schleiper (1929 *a* and *b*), Adolph (1925), Goldfarb (1914) and many others have found that salinity also affects the weight of an animal. Schleiper (1929 *a* and *b*) and Beadle (1931) show that such alterations are followed by changes in the respiratory rate. The effect of the environment is collective and all factors such as Temperature, Excess Base, Salinity, pH, Nitrate, Silicate and Ammonia content may influence in some way or other the metabolism of an animal.

The authors of the present paper have studied the environmental conditions and have attempted to correlate these with changes in the size, quantity, and variation of the inclusions observed by them in a few animals. Whether these changes are caused by any one factor or whether they are due to all of them is difficult to prove.

Methods and Materials.

Clibanarius olivaceus was obtained from the brackish waters at the mouth of the river Adyar and *Salmacis* and *Dasychone* from the Madras Harbour. Water samples on the days of collection were analysed and the following methods have been employed.

Hydrographical Technique.—

(1) All water analysis records given are of samples collected at 8 a.m. from the mouth of the river, as well as from the Harbour, irrespective of the nature of the tide.

(2) The temperature was determined by the aid of a surface temperature thermometer giving readings correct to 0.1°C. Each reading is the mean of three readings taken from places at approximately 50 yards from each other.

(3) The pH was determined with the aid of a Hellige Comparator.

(4) For the determination of salinity, both Knudsen's Chlorine Titration apparatus and Richter and Weiss' set of aerometers were used.

(5) The Excess Base was determined according to the method outlined by Bruce (1924) and the results obtained were checked from time to time by Saunders's method (1926).

Cytological Technique.—The material was fixed immediately it was brought into the Laboratory. The ovaries were removed—out of contact

Records of Sea and River Water Analysis.
December 1932—*July* 1933.

A	B Temperature		C pH		D Excess Base		E Salinity		F Chlorine		G Air Temperature	Difference between Air and Sea Temperature	Difference between Air and River Temperature
Month	Sea	River	Sea	River	Sea	River	Sea	River	Sea	River			
December ..	75·9°F.		8·41		18·7		28·73		15·90				
January ..	76·01	77·0	8·47	8·48	19·98	20·73	30·59	28·17	16·93	15·59	75·2°F.	plus 0·81	plus 1·8
February ..	79·16	79·16	8·5	8·55	20·25	23·2	31·94	30·44	17·68	16·85	75·6	„ 3·56	„ 3·56
March ..	82·94	81·86	8·49	8·41	19·55	17·59	33·88	29·92	18·75	16·56	78·3	„ 4·64	„ 3·56
April ..	83·12	82·76	8·5	8·46	20·2	25·16	34·48	17·29	19·09	9·56	84·5	minus 1·38	minus 1·74
May ..	85·28	85·37	8·65	8·65	19·9	24·9	34·81	19·74	19·27	10·92	86·8	„ 1·52	„ 1·43
June ..	83·48	83·84	8·48	9·07	19·78	24·52	35·39	16·91	19·57	9·35	84·5	„ 1·02	„ 0·66
July ..	83·48		8·43		20·72		35·16		19·46				
Total No. of Readings ..	16	15	29	24	25	22	18	15	18	15			

with any liquid—cut into small pieces and immediately transferred to the fixatives. Carnoy, Flemming, Flemming without acetic, Champy Kull, Regaud, Bensley-Cowdry, Nassonov's modification of Kolatschev's method, Mann Kopsch, Mann Kopsch Altmann, Ludford's modification of Mann Kopsch, Da Fano, Corrosive acetic and absolute alcohol were employed. The stains generally used were Heidenhain's Iron-Alum Hæmatoxylin, Orange G, Alum Carmine, Acid Fuchsin, Toluidin blue, Aurantia, Thionin, Methyl Green, Methyl Blue Eosin, Pyronin Methyl Green, etc.

In addition fresh eggs were studied before and after treatment with osmic acid. Ciaccio's test for lipoids as well as Scharlach R and Sudan III stains were also used.

From regular records of analyses of the Sea and River water, are given below the results for the months December to July. (See Table I.)

Conditions in the Brackish Water.

The conditions observed at the mouth of the river differ entirely in the two seasons. In January-February after the heavy rains of the South-West Monsoon, the bar is open, and the sea water mixes with the river water during high tide. As can be gleaned from the Table, the temperature of the water is near the minimum during these periods and the salinity is very high. The pH is also low in comparison with that for the April-June period and the same may be said of Excess Base. The readings for March—except in the case of temperature—are lower owing to the unusual rain of 3·42″ recorded for that month in Madras.

Conditions in the Period Mid-April to June.

From the middle of March the level of water in the river begins to fall with the consequent widening of the bar. The open river mouth becomes narrower and narrower and also silts up, and in the first week of April the sea invades the river only at high tide. Even this ceases in the middle of April, resulting in an entire change in salinity.

With the closing of the river, the salinity of the river water shows a sudden fall from 29·92 to 17·29. Changes also take place in the Excess Base and pH.

As can be seen from the Table the air temperature is near the maximum for the year in the months of April, May and June.

Conditions in the Sea.

The conditions observed in the Madras Harbour are, however, different. The temperature shows a steady rise from January to July. The pH values

fluctuate slightly, the minimum observed being in December and the maximum in May. In general it may be said that there is a steady increase from December to May after which there is a fall. The Excess Base shows no orderly rise probably due to the peculiar conditions existing in the Harbour. The salinity is very low in December as a result of the mixing of fresh water brought in by the river but shows a steady rise till June when it reaches the maximum. It will be noticed that in comparison with the records for the brackish water the 3·42″ of rain affects only the pH and Excess Base readings.

The temperature of the sea also follows the same course as that of the river being above that of the air in January, February and March and below in April, May and June.

Sizes of Eggs during the Two Seasons.

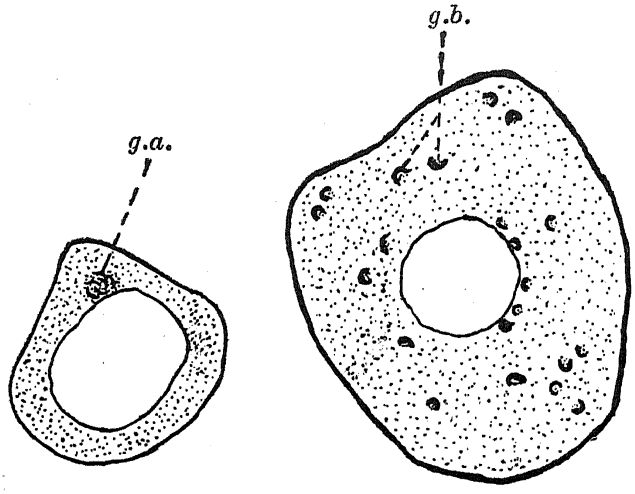
	Clibanarius		*Dasychone*		*Salmacis*	
	Jan.-Feb.	Apr.-Jun.	Dec.-Jan.	May.-Jun.	Dec.-Jan.	Apr.-Jun.
Average Size	302·8 μ	391·6 μ	85·6 μ	77·5 μ	102·91 μ	100·87 μ
Smallest fully developed egg..	246·6 μ	277·2 μ	69·3 μ	62·7 μ	79·2 μ	72·6 μ
Biggest egg	518·8 μ	569·8 μ	102·3 μ	99·0 μ	138·6 μ	122·1 μ

Bi-weekly measurements of 250 eggs on an average were continued throughout the period of investigation. It will be seen from the Table that whereas in the case of the marine animals there is a consistent decrease in size, in *Clibanarius* which is a brackish water form there is an increase. All these animals are exposed to the same high temperatures and hence the temperature factor may be eliminated. The notable changes are only in salinity and while in the sea the maximum is reached in May-June, in the river there is a sudden fall. It is presumed from the above results that the variation in the sizes of the eggs is caused by variations in salinity.

Oogenesis of Dasychone.

The Golgi Apparatus.—In the youngest oocytes studied the Golgi apparatus occurs as a few batonettes on one side of the nucleus (Fig. 1). In Fig. 2 is shown the spreading of the Golgi batonettes from this initial concentration. In December-January preparations individual batonettes migrate to various parts of the cytoplasm but in those in May-June (Fig.3) clumps of two or three first break off and the individuals constituting these later separate and become scattered throughout the cytoplasm. From the

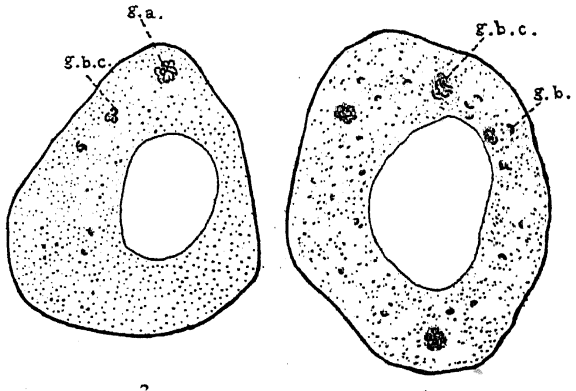
commencement of migration, these batonettes increase in number as could be made out from Figs. 1, 2 and 3. It has not been possible to ascertain whether the multiplication is by transverse or longitudinal fission but the orientation of these bodies suggests that it is longitudinal. Both in December-January and May-June oocytes multiplication appears to be rapid from the time the batonettes get scattered throughout the cytoplasm. In December-January especially the batonettes occur in clumps as shown in Fig. 4.



1. 2.

FIG. 1. Earliest stage of Golgi bodies. December-January. Da Fano. × 2,000.

FIG. 2. Spreading of the dictyosomes throughout the cytoplasm. December-January. Da Fano. × 2,000.



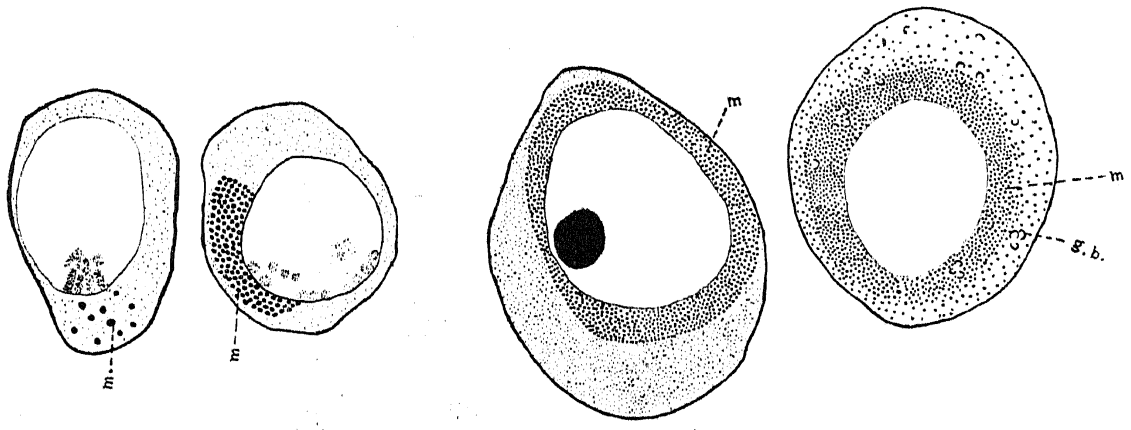
3. 4.

FIG. 3. Spreading of the dictyosomes in May-June. Da Fano. × 1,500.

FIG. 4. Multiplication of the dictyosomes. December-January. Da Fano. × 1,000.

The Mitochondria.—In Fig. 5 which is that of a very young oocyte, is shown the mitochondria, occurring as a few large grains on one side of the nucleus. These divide, become smaller and begin to spread around the

nucleus. In Figs. 6 and 7 are shown the formation of a circum-nuclear concentration by the mitochondria. The depth of this concentration increases probably due to division and the grains begin to migrate to the other portions of the cytoplasm (Fig. 8). The spreading is uniform and takes place all



5. 6. 7. 8.

FIG. 5. Earliest stage of mitochondria. *F. w. a.* Iron Hæmatoxylin. December-January. × 1,500.

FIG. 6. Multiplication stage of mitochondria. *F.w.a.* Iron Hæmatoxylin. December-January. × 1,500.

FIG. 7. Formation of a circum-nuclear concentration by the mitochondria. *F.w.a.* Iron Hæmatoxylin. December-January. × 1.500.

FIG. 8. Spreading of the mitochondria from the circum-nuclear concentration. Flem. *F. w. a.* Iron Hæmatoxylin. December-January. × 1,000.

over. In the fully grown oogonium (Fig. 9) the mitochondria are scattered uniformly throughout the cytoplasm.

In May-June preparations the mitochondria behave similarly but from the time of commencement of migration from the circum-nuclear concentration a sudden increase in size has been noticed. Figs. 9 and 10 which are camera lucida drawings under the same magnification show the difference in size. The mitochondria in May-June are almost ten times as large as those in December-January.

The Nucleolus.—The nucleolus occurs as a spherical body in the young oocytes (Fig. 7). It is not well fixed at all in December-January Da Fano and Bouin material. In May-June the distortion is comparatively less. There appear to be individual variations also. The nucleolus is darkened by osmic fixatives in both seasons but the blackening in December-January material can be extracted in a few minutes with turpentine. In May-June, extraction is negligible even after 6 days. The nucleolus begins to bud as

shown in Fig. 9. The mode of budding also exhibits individual variations. Only one or two buds at a time may be formed or a large number may be

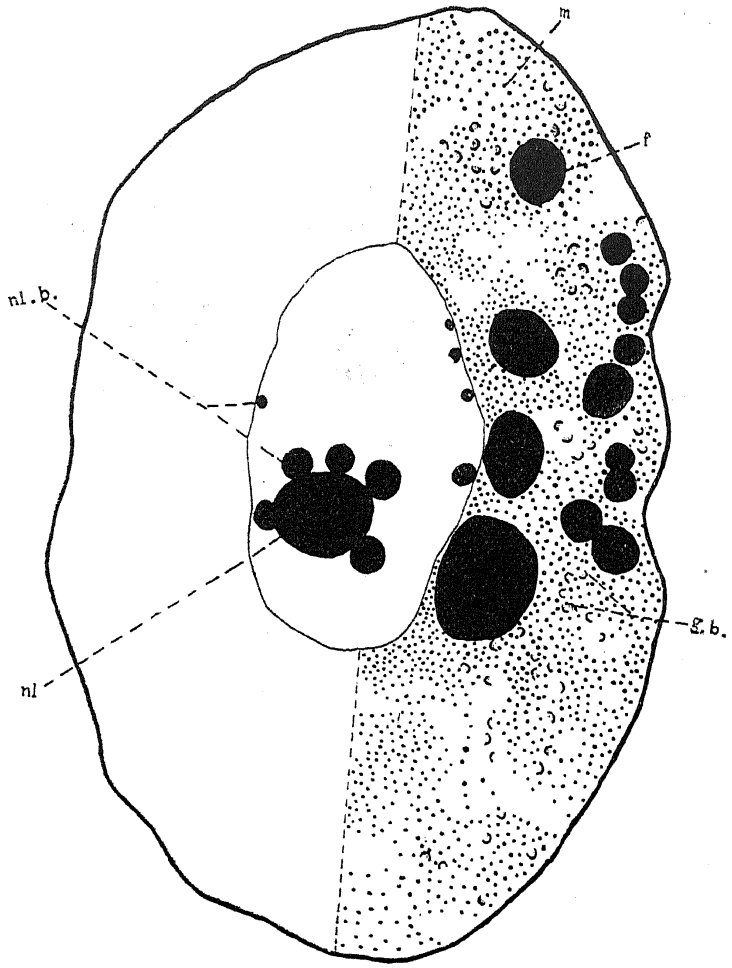
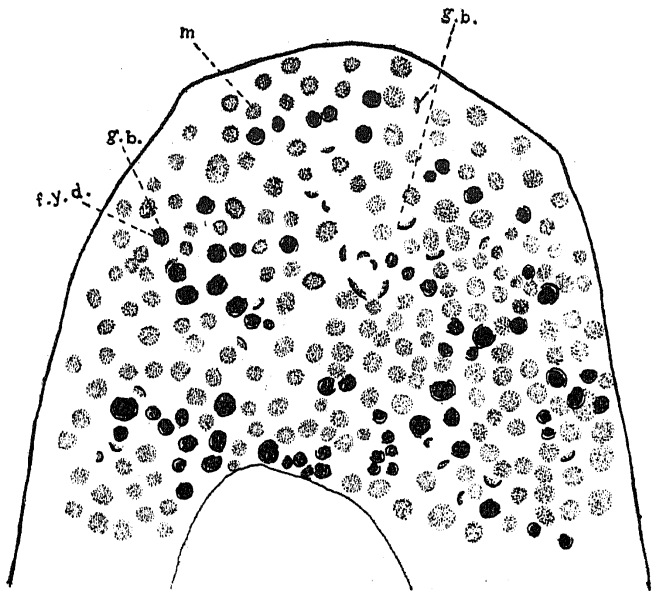


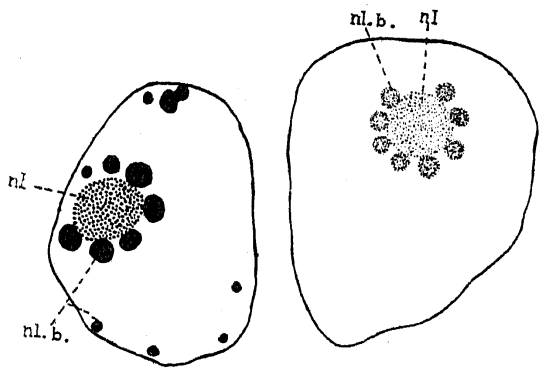
FIG. 9. An almost fully grown egg showing dictyosomes, fat vesicles, nucleoli and mitochondria. *F. w. a.* Iron Hæmatoxylin. December-January. × 1,000.

produced all round the nucleolus (Figs. 9, 11, 12). These buds also exhibit reactions similar to those of the original nucleolus but with this difference ; in December-January the buds are not fixed at all in Bouin and Da Fano and the nucleolus is very much distorted, whereas in May-June the distortion is less and the buds are often preserved though much changed in appearance. Reactions of these buds and nucleoli to Sudan III and Scharlach R also differ in the two seasons. In December-January both are stained deep red (Fig. 11) while in May-June they (Fig. 12) stain orange of slightly varying shades. The nucleolar buds detach themselves from the main mass and pass to the nuclear membrane to which they firmly stick (Figs. 9 and 11). No rupture of the nuclear membrane has been observed but as the fat and fatty yolk is



10.

FIG. 10. A portion of an almost fully grown egg from a May-June preparation and showing the larger size of the mitochondria, two types of Golgi bodies, fatty yolk etc. Nassonov unstained. × 1,000.



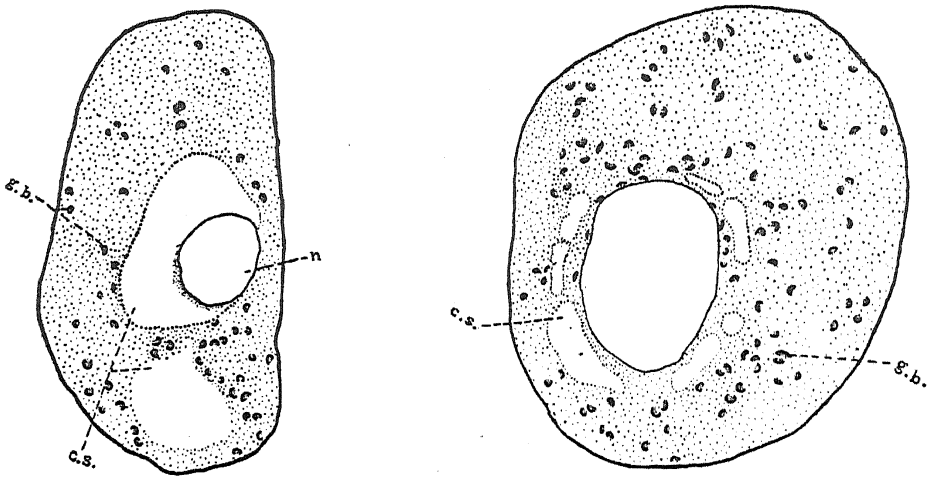
11. 12.

FIG. 11. Nucleus with the contained nucleoli. Both the nucleolus and the nucleolar buds are stained deep red by Sudan III and Scharlach R December-January. × 1,000.

FIG. 12. Indra vital staining of fresh eggs with Sudan III and Scharlach R in May-June. Nucleolus and nucleolar buds are stained in varying shades of orange. × 1,000.

formed near the nucleus the authors believe that the materials in the buds diffuse into the cytoplasm through the nuclear membrane.

Fat and Fatty Yolk.—It will be seen that in comparing Figs. 3, 4 and 13 there is a sudden decrease in the number of Golgi bodies (see Fig. 13) followed by the appearance of clear spaces. Both being Da Fano the possible explanation for such a phenomenon is that some of the Golgi have been converted into fat. These spaces also increase in number and size with the growth



13. 14.

FIGS. 13 and 14. Fat formation. Note the clear spaces left by the fat vacuoles. Da Fano, December-January. × 600.

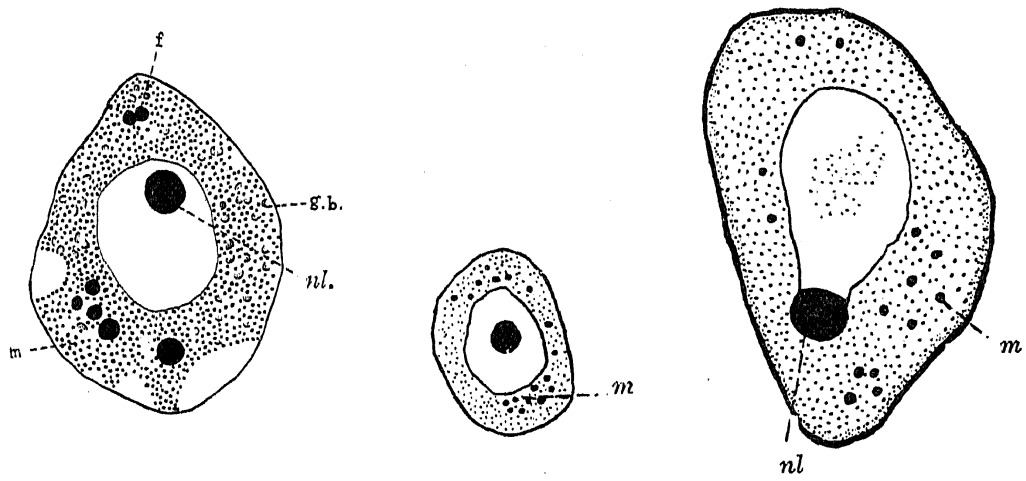
of the oocyte as can be seen from Figs. 13 and 14. Fig. 15 is from a Nassonov preparation and shows the first appearance of fat globules. These globules are black in osmic fixatives and are not preserved in Bouin, Corrosive acetic, Da Fano, etc. The sudden decrease in the number of Golgi suggests a transformation of the Golgi into fat and we are led to believe that each droplet of fat is formed by a group of Golgi bodies. The unmodified Golgi continue the division process started before and more and more Golgi bodies get transformed into fat. The diffusion of the nucleolar material begins after the division of the Golgi bodies comes to a standstill. The fat globules are near the nuclear membrane and the diffusion of nucleolar material is followed by an increase in the size of the fat globules. That some new vacuoles are also formed from the material of the nucleolar buds is suggested by the very great disparity in the size of the fat globules as also by the late origin of some of the smaller fat vacuoles. In the mature oogonium (Fig. 9) there are Golgi bodies, fat droplets and mitochondria in the December-January period. The fat droplets blackened after Champy and F.w.a. are dissolved out by the xylol in Canada balsam in the course of a fortnight after which they appear as clear vacuoles. In Nassonov and Mann Kopsch preparations such a quick dissolution is not apparent but even here the droplets look dull black after six months. Sudan III and Scharlach R stain these deep red and Ciaccio's test gives an orange colour for the Golgi and a red one for fat vacuoles.

Fatty Yolk.—In May and June, examination of fresh eggs, as well as fixed preparations, reveals the absence of any fat globules. There are only two inclusions in the young oocytes, the Golgi and the mitochondria. When nucleolar emissions begin, the Golgi which up till now were uniform in size

begin to get differentiated into two sets. Some grow larger in size than the others. In fresh eggs and fresh eggs osmicated for a short time the following reactions have been observed. In December-January oocytes and oogonia and in May-June earliest oocytes the chromophobe part of the batonettes becomes visible only after varying periods of osmication, being not visible in fresh eggs. In both cases the chromophobe part does not give any reaction to Ciaccio's test. But when in May-June growing oocytes, the Golgi begins to differentiate into two sets, namely larger and normal ones, in the former the chromophobe part becomes visible *intra vitam*. From the time of the beginning of differentiation Sudan III and Scharlach R stain this part orange. In osmic fixatives they are blackened (ref. Fig. 10 *f.y.d.*) but as in the case of the nucleolus and nucleolar buds in May the colour is not extracted by turpentine even after six days. In Champy and F.w.a. material xylol does not decolourise these portions. We consider these as the fatty yolk droplets, lipoidal in composition and infer that the material of these droplets is derived from the nucleoli which have diffused out and have been condensed by the Golgi. There is no transformation of Golgi.

Seasonal Variation in Salmacis.

In a previous paper one of us (Subramaniam, 1934) studied the oogenesis of *Salmacis bicolor*. The study was extended over a period of two seasons. The most striking facts observed were (1) a variation in the sizes of the oocytes when the mitochondria were first visible (Figs. 16 and 17)



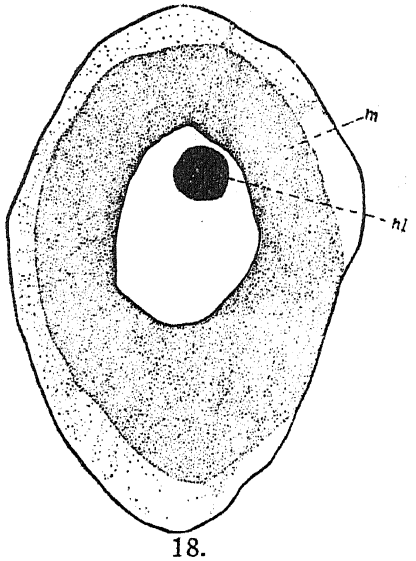
15. 16. 17.

Fig. 15. Beginning of fat formation. Nassonov. December-January. × 1,000.

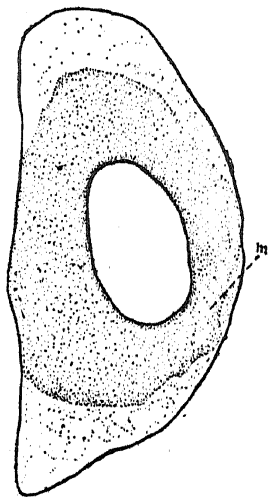
Fig. 16. *Salmacis*. Earliest stage of mitochondria. December-January. Flemming without acetic. Iron Hæmatoxylin. × 3,000.

Fig. 17. *Salmacis*. Earliest stage of mitochondria observed in preparations made in April. Flemming without acetic. Iron Hæmatoxylin. × 3,000.

and (2) a variation in the quantity of the mitochondria as also of yolk (Figs. 18 and 19). The nutritive bodies formed are fewer in April-May and a comparison of the figures 18 and 19 will show that the mitochondrial circum-nuclear concentration is very thin as against the very thick one in



18.



19.

FIG. 18. *Salmacis*. A stage just before the cytoplasm of the oocyte is filled up by the spreading mitochondria. An attempt has been made to represent the thickness of the mitochondrial concentration. *F. w. a.* Iron Hæmatoxylin. December-January. × 600.

FIG. 19. *Salmacis*. An oocyte at the same stage as in Fig. 18. Note the sparse distribution of the mitochondria. *F. w. a.* Iron Hæmatoxylin. × 600.

December-January. Yolk spherules formed are also very much less in May in correlation with the smaller quantity of the mitochondria and nutritive spheres.

Seasonal Variation in Clibanarius.

Oogenesis of *Clibanarius olivaceus* (Henderson) worked out by Subramaniam (1935 *a*) has revealed the existence of a difference in the mode of formation of albuminous yolk and in the amount of both fatty and albuminous yolk. In January-February oocytes there is a voluminous formation of fatty yolk and albuminous yolk of Golgi-mitochondrial origin alone has been observed. In April-June on the other hand fatty yolk formation is less and that of albuminous yolk more and two varieties of albuminous yolk—Golgi-mitochondrial and Golgi-Golgi—occur.

Discussion.

It will be seen from the material presented in this paper that the environmental factors as they change affect the size, quantity, behaviour and time of appearance of these inclusions. Work on *Meretrix casta** which has already

* *Journal of Morphology* (in press).

been completed in this Laboratory shows that variations in the behaviour and manner of spreading of the Golgi bodies are considerable. In addition, in the three forms described in this paper we get a pronounced seasonal variation which is all the more remarkable.

This leads to the general questions (1) why such variations occur during different seasons of the year and (2) whether similar variations may be expected in animals collected from different parts of the world.

We are led to believe that these variations in the behaviour and quantity of the inclusions in correlation with seasonal changes in the physico-chemical factors of the medium represent variations in metabolism. The observations recorded in the case of *Clibanarius, Dasychone* and *Salmacis* allow of no other interpretation. This is further supported by the results obtained by Bogorov (1934), Bogorov and Preobrajensky (1934), Moore (1934), Orr (1934), Marshall, Nicholls and Orr (1934), and Russell (1928). To the cytologist the cell is the unit and variations in the metabolism in the animals are only expressions of the changes in the cell groups under different conditions. Does not this interpretation explain the somewhat diverse results obtained by workers on the same animal collected from the same locality ?

Bogorov's and Bogorov and Preobrajensky's results throw some light on the cause of the varying results obtained by workers in different parts of the world. Further evidence in support of our view, *viz.*, that differences in the environment, whether seasonal or geographical, are probably due to changes in the metabolism of the animals, is provided by the different conclusions arrived at by different workers in the case of *Lithobius*.

Reverting now to the cell, recent work has shown clearly that the mitochondria and the Golgi bodies are concerned in the yolk or fat formation in the eggs of various animals. If the Golgi bodies are the active agents in the synthesis of fat or yolk and if such a conclusion is based on topographical as well as diagnostic characters, it is reasonable to expect a similar function in cells where there is no fat or yolk. The scattering of the parts of the Golgi apparatus during cell division has been taken to represent an increase in the metabolism of the cell, and hence the uniform distribution of the Golgi bodies from an initial concentration in the egg in which there is no yolk or fat, indicates an attempt by the apparatus to cope with the increasing metabolism of the cell. How metabolism in the cell is controlled has baffled all interpretation. Theoretical as well as experimental evidence (Subramaniam, 1934, 1935 *a*, 1935 *b*, 1935 *c* : oogenesis of *Meretrix casta* (in press) ; Subramaniam and Gopala Aiyar, 1935 *a* and 1935 *b*) has been offered in previous papers for concluding that the Golgi apparatus is the fountain-head of the various enzymes. And, on the basis of such an explanation, it becomes

increasingly evident why there should be variations in the behaviour of the apparatus with changes in metabolism.

Summary.

1. The oogenesis of *Dasychone* and *Salmacis* has been worked out from oocytes collected during two different seasons.

2. Changes in the physico-chemical factors of the Sea and the Adyar river water have been studied.

3. Measurements of the ripe oocytes indicate a consistent increase in size in the oocytes of *Clibanarius* and a decrease in *Dasychone* and *Salmacis* from January to June. It is presumed that these alterations in size are caused by variations in salinity.

4. In December-January preparations of *Dasychone*, fat—the only deutoplasmic inclusion—appears to be formed by the transformation of Golgi material.

5. In May-June fatty yolk alone has been observed in the eggs and this appears to be secreted by the dictyosomes.

6. The nucleoli and nucleolar buds are stained deep red in December-January while in May-June they are orange, of slightly varying shades.

7. In *Salmacis* also, there is a variation in the quantity of nutritive bodies, mitochondria and yolk in the two seasons.

8. An attempt has been made to show that the remarkable diversity of results obtained by workers on the same animal are due to variations in the environment: (1) seasonal, (2) geographical.

KEY TO LETTERING OF ALL TEXT-FIGURES.

c.s. Clear spaces left by fat vacuoles in Da Fano sections ; *f.* Fat vacuoles; *f.y.d.* Fatty yolk vacuoles ; *g.a.* Golgi apparatus ; *g.b.* Golgi batonettes ; *g.b.c.* Golgi batonettes occurring as a clump ; *m.* Mitochondria ; *n.* Nucleus ; *nl.* Nucleolus ; *nl.b.* Nucleolar buds.

BIBLIOGRAPHY.

Adolph, E. F. .. .. "Some Physiological Differences between Fresh water and Marine Organisms," *Biol. Bull.*, 1925, **48.**

Atkins, W. R. G. .. .. "Hydrogen Ion Concentration of Sea Water in its Biological relations," *Jour. Mar. Assn.*, 1922, **12.**

Beadle, L. C. .. .. "The Effect of Salinity on water content and respiration of marine Invertebrates," *Jour. Expl. Biol.*, 1931, **8.**

Bogorov, B. G. .. .. "Seasonal Change in the Biomass of *Calanius finmarchicus* in the Plymouth area in 1930," *Jour. Mar. Biol. Assn.*, May, 1934, **19.**

Bogorov, B. G., and Preobrajensky, E. N. "The Biomass of Plankton Organisms of the Barent's Sea. II. Copepoda." (Quoted by Bogorov.)

Brandt, K. .. .. "Beitrage zur Kenntniss der Chemischen Zussammensetzung des Planktons," *Wiss. Meeresunters. Abt. Kiel.*, 1898, **9**.

Brandt, K., and Raben, E. .. "Zur Kenntniss der Chemischen Zussammensetzung des Planktons und einiger Boden-organismen," *Wiss. Meeresunters. N. F. Abt. Kiel.*, 1919, **19**.

Bruce, J. R. .. .. "A pH method of determining the Carbondioxide exchanges of Marine, Brackish water and Fresh water Organisms," *Jour. Expl. Biol.*, 1924, **2**.

Bruce, J. R. .. .. "Physical Factors on the Sandy Beach. I. Tidal, Edaphic and Climatic," *Jour. Mar. Biol. Assn.*, 1928, **15**.

Bruce, J. R. .. .. "Part II. Chemical Changes, Carbon dioxide concentration and sulphides," *Jour. Mar. Biol. Assn.*, 1928, **15**.

Brambell, F. W. R. .. "The Activity of the Golgi apparatus in the Neurones of Helix aspersa," *Jour. Phy.*, 1923, **57**.

Calkins, G. N. .. .. "Observations on the Yolk Nucleus in the egg of *Lumbricus*," *Trans. N. Y. Acad. Sci.*, 1895.

Foot, K. .. .. "Preliminary observations on the maturation and fertilization of the egg of *Allolobophora fœtida*," *Jour. Morph.*, 1894, **9**.

Foot, K. .. .. "Yolk Nucleus and Polar Ring," *Jour. Morph.*, 1896, **12**.

Foot, K., and Strobell, E. C. "Further Notes on the egg of *Allolobophora fœtida*," *Zool. Bull.*, 1898, **2**. (Quoted by other authors.)

Foot, K., and Strobell, E. C. "Photographs of the egg of *Allolobophora fœtida*," *Jour. Morph.*, 1900, **16**.

Foot, K., and Strobell, E. C. "Photographs of *Allolobophora fœtida*, II," *Jour. Morph.*, 1901, **17**.

Gatenby, J. B. .. "The Cytoplasmic Inclusions of Germ Cells, V. The Gametogenesis and Early development of *Limnæa stagnalis* with special reference to the Golgi apparatus and the mitochondria," *Quart. Jour. Micr. Sci.*, 1919, **63**.

Gatenby, J. B. .. .. "The Cytoplasmic Inclusions of Germ Cells. Part X. Gametogenesis of *Saccocirrus*," *Quart. Jour. Micr. Sci.*, 1922, **66**.

Gatenby, J. B., and Nath, V. "The Oogenesis of certain Invertebrata with special reference to *Lumbricus*," *Quart. Jour. Micr. Sci.*, 1926, **70**.

Goldfarb, A. J. .. .. "Changes in Salinity and effects on Regeneration," *Carnegie Instn. Publn. Wash.*, 1914, **6**.

Harvey, L. A. .. .. "On the relationship between mitochondria and the Golgi apparatus to yolk formation in the egg of the common earthworm *Lumbricus terrestris*," *Quart. Jour. Micr. Sci.*, 1925, **69**.

Harvey, L. A. .. .. "The History of the Cytoplasmic Inclusions of the egg of *Ciona intestinalis*, during oogenesis and fertilization," *Proc. Roy. Soc.*, London, B., 1927, **101**.

Harvey, L. A. .. .. "The Oogenesis of *Carcinus mœnas* (Penn) with special reference to Yolk formation," *Trans. Roy. Soc. Edin.*, 1929, **56**.

Harvey, L. A. .. .. "Oogenesis of *Lumbricus*, A Restatement," *Quart. Jour. Micr. Sci.*, 1931*a*, 74.

Harvey, L. A. .. .. "Studies on Echinoderm Oogenesis. I. *Antedon bifida*," *Proc. Roy. Soc.*, London B., 1931*b*, 107.

Harvey, L. A. .. .. "Studies on Echinoderm Oogenesis, II. *Asterias rubens* (Linne)," *Proc. Roy. Soc.*, London, B., 1931*c*, 107.

Harvey, H. W. .. .. "Biological Physics and Chemistry of Sea Water," *Camb. Phy. Ser.*, 1928.

Hirschler, Jan .. .. "Uber die Plasmakomponenten der weiblichen Geschlechtzellen," *Arch. mikr. Anat.*, 1917, 89.

King, S. D. .. .. "Oogenesis of *Lithobius forficatus*," *Proc. Roy. Dub. Soc.*, 1924, 18.

Koch, A. .. .. "Morphologis des Einwachstums der Chilopoden," *Zeit. zellf. mikr. Anat.*, 1925, 2.

Lieber, Alice .. .. "Zur Oogenese einiger Diopatrarten," *Zeit. fur. wiss. Zool.*, 1931, 138.

Ludford, R. J. .. .. "The behaviour of the Golgi bodies during nuclear division with special reference to amitosis in *Dytiscus marginalis*," *Quart. Jour. Micr. Sci.*, 1922, 66.

Ludford, R. J. .. .. "The General and Experimental Cytology of Cancer," *Jour. Roy. Micr. Soc.*, 1925.

Marshall, S. M., Nicholls A.G., and Orr, A. P. "On the Biology of *Calanus finmarchicus*. V. Seasonal distributions, Size, Weight and Chemical composition in Loch Striven in 1933 and their relation to Phytoplankton," *Jour. Mar. Biol. Assn.*, 1934, 19.

Moore, H. B. .. .. "A comparison of the Biology of *Echinus esculentus* in different habitats," *Jour. Mar. Biol. Assn.*, 1934, 19.

Nath, V. .. .. "Studies on the shape of the Golgi apparatus. II. Observations on the fresh egg of the Indian Earthworm *Pheretima posthuma*," *Quart. Jour. Micr. Sci.*, 1930, 73.

Nath, V. .. .. "Microchemical Tests for Fats, Lipoids and Vacuoles with special reference to Oogenesis," *Quart. Jour. Micr. Sci.*, 1933, 76.

Orr, A. P. .. .. "On the Biology of *Calanus finmarchicus*. IV. Seasonal Changes in the Weight and Chemical composition in Loch Fyne," *Jour. Micr. Biol. Assn.*, 1934, 19.

Orton, J. H. .. .. "Sea Temperature, Breeding and Distribution in Marine Animals," *Jour. Mar. Biol. Assn.*, 1922, 12.

Pantin, C. F. A. .. .. "Adaptation of *Gunda ulvae* to Salinity. I. The Environment," *Jour. Expl. Biol.*, 1931, 8.

Pantin, C. F. A. .. .. "Adaptation of *Gunda ulvae* to Salinity. III. The Electrolyte Exchange," *Jour. Expl. Biol.*, 1931*b*, 8.

Rau, A. S., and Ludford, R. J. "Variation in the form of the Golgi bodies during the development of Neurones," *Quart. Jour. Micr. Sci.*, 1925, 69.

Russel, F. S. .. .. "The vertical Distribution of Marine Macroplankton. VII. Observation on the behaviour of *Calanus finmarchicus*," *Jour. Mar. Biol. Assn.*, 1928, **15**.

Saunders, J. T. .. .. "The Hydrogen Ion Concentration of Natural Waters. 1. The relation of pH to the pressure of Carbon dioxide," *Jour. Expl. Biol.*, 1926, **4**.

Schleiper, C. .. .. "Einwirkung niederer Salzkonzentration auf marine organismen," *Zeit. f. vergil. Physiol.*, 1929*a*, **9**. (Quoted by Beadle and Pantin.)

Schleiper, C. .. "Neue Versuche uber die Osmoregulation wasserlebender Tiere," *Sitzungbericht de Ges. z. Beford d. gesamt. Naturwiss. Marburg.*, 1929*b*, **64**. (Quoted by Pantin and Beadle.)

Stott, F. C. .. .. "The spawning of *Echinus esculentus* and some changes in Gonad composition," *Jour. Expl. Biol.*, 1931, **8**.

Subramaniam, M. K. .. "Oogenesis of *Salmacis bicolor* (Agassiz) with a suggestion as to the function of Golgi bodies," *Proc. Ind. Acad. Sci.*, Bangalore, 1934, **6**.

Subramaniam, M. K. .. "Oogenesis of *Clibanarius olivaceus* (Henderson) with special reference to a seasonal variation in the cytoplasmic inclusions," *Jour. Roy. Micr. Soc.*, 1935*a*.

Subramaniam, M. K. .. "Preliminary observations on the effect of Fertilization on the Golgi bodies in the egg of *Acentrogobius neilli*," *Proc. Ind. Acad. Sci.*, 1935*b*, **1**, No. 10.

Subramaniam, M. K. .. "A Cytological Study of the structure and formation of Nutritive bodies in *Salmacis bicolor*," *Zeit. fur. Wiss. Zool.*, 1935*c*, **146**.

Subramaniam, M. K., and Gopala Aiyar, R. "Oogenesis of *Acentrogobius neilli* (*Gobius neilli*, Day) with special reference to the behaviour of the Nucleoli," *Jour. Roy. Micr. Soc.*, 1935*a*, **55**.

Subramaniam, M. K., and Gopala Aiyar, R. "Secretion of Fatty and Albuminous Yolk by Golgi bodies in *Stomopneustes variolaris*," *Zeit. zellf. u. mikr. Anat.*, 1935*b*, (In press.)

Tennent, D. H., Gardiner, M. S., and Smith, D. E. "A Cytological and Biochemical Study of the Ovaries of *Echinometra lucunter*," *Carnegie Instn. Wash. Pubn.*, 1931, **27**, No. 413.

Weil, A., and Pantin, C. F. A. "Adaptation of *Gunda ulvae* to Salinity. II. Water Exchange," *Jour. Expl. Biol.*, 1931, **8**.

Weiner, P. .. .. "Contributions a l'etude des noyaux vitellins," *Arch. Russ. Anat. Embryol.*, 1925, **4**. (Quoted by other authors.)

Weiner, P. .. .. "Vitale Beobachtungen uber den Golgi apparat bei der Oogenese der Regenwurmer Allolobophora calliginosa und Eisenia rosea," *Zeit. mikr. Anat. forsch.*, 1930, **20**.

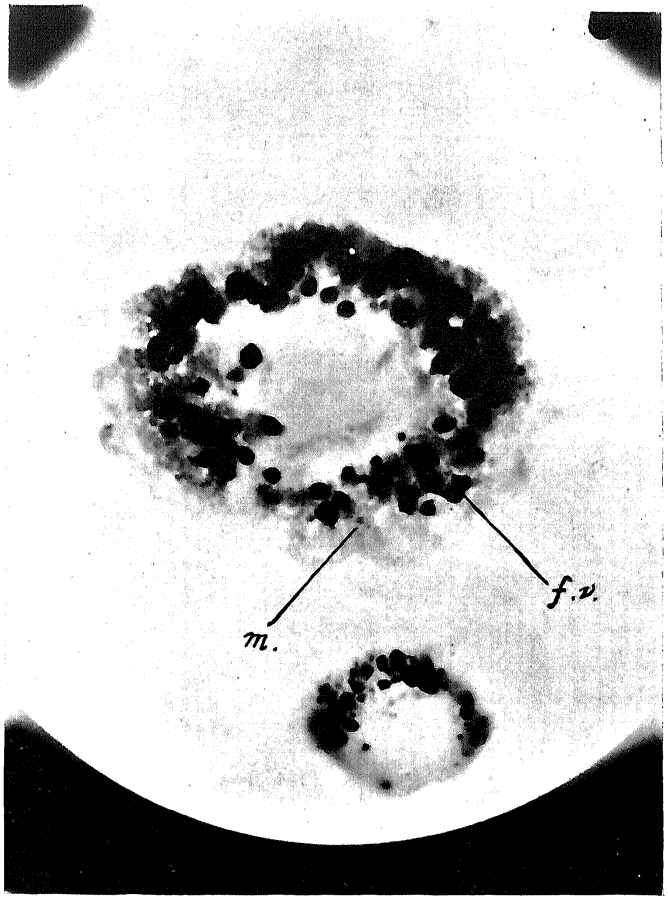
Wimpenny, R. S. .. "Preliminary observations on the fat content of the Plankton of the English Herring Grounds in the North Sea," *Ministry of Agriculture and Fisheries, Fisheries Investigations. Ser. II.*, 1929, **2**, No. 5.

Addendum.

In some of the *Dasychone* material fixed in November 1935 we came across eggs in which fat and fatty yolk occur side by side. This intermediate condition gives additional proof of the existence of a seasonal variation.

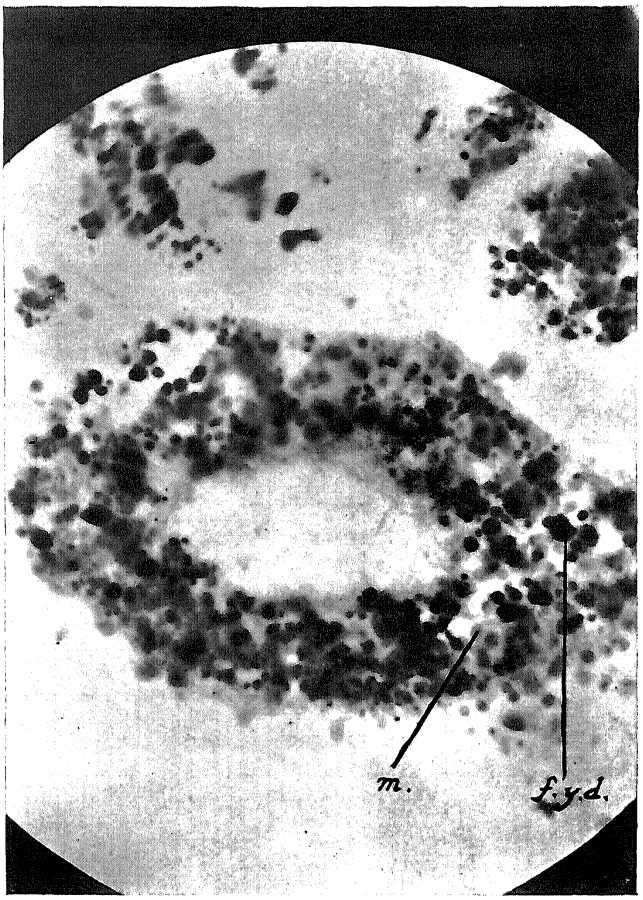
Photomicrograph 1 shows an egg with fat droplets (*f. v.*) and granular mitochondria (*m*) taken from a preparation made in January-February 1933. In photomicrograph 2—a May-June preparation of the same year—can be seen fatty yolk vesicles (*f. y. d.*) and swollen mitochondria. The transitional stage can be seen in photomicrograph 3 (November 1935) where the mitochondria are becoming granular and where fat and fatty yolk occur side by side. Unfortunately no preparations were made in November 1933.

1



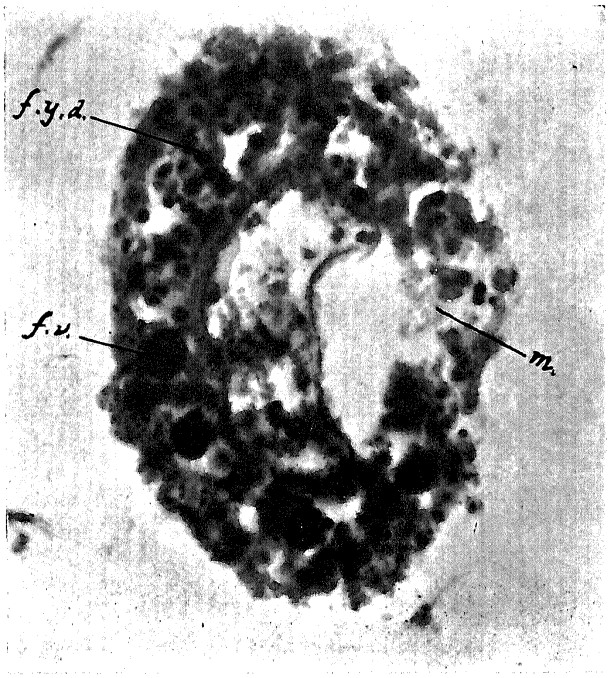
Jan.—Feb. 1933

2



May—June 1933

3



Nov. 1935