2. Cytasters and Centrosomes in Artificial Parthenogenesis.

By Edmund B. Wilson.

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In a recent paper on artificial parthenogenesis by Dr. A. Petrunkewitsch[1] doubt is cast upon my results regarding the origin of the centrosomes in artificially induced parthenogenesis in sea-urchins, an endeavour being made to show that the centrosomes of the multiple asters arise, not by new-formation as I concluded, but by progressive division from the primary egg-center. This result has been based on an evidently conscientious research, and is supported by figures that may seem convincing to those who are not sufficiently familiar with the object or not fully acquainted with the evidence. The importance of the question at issue leads me therefore to point out on how inadequate, and in some respects misleading, a basis the conclusions of Dr. Petrunkewitsch rest.

The author seeks to establish the conclusion that those asters which contain central bodies (»echte Strahlungen«) are of wholly different nature from the smaller ones that do not contain such bodies (to which alone he would apply the term "cytasters"), and finds that the former arise only by progressive division from the primary egg-center, and do not form at all in enucleated egg-fragments — »daß eine Neubildung von Centrosomen nie stattfindet, daß vielmehr allen neugebildeten echten Strahlungen als Zentren echte Centrosomen dienen, die durch die Theilung des Eicentrosomas entstanden sind« (S. 35). In preparations, »können wir die ganze Entwicklung der Strahlungen Schritt für Schritt verfolgen, wir können die Entstehung der Centrosomen aus dem Eizentrum nachweisen« usw. (op. cit. S. 37). But the series of figures (Figs. 15—20), given in support of this rather sweeping assertion, affords no real basis for such a conclusion, and in the light of my observations on the living eggs is open to an entirely different interpretation. This series of figures is in fact constructed by the selection of preparations of fixed material; and that they represent successive stages of development is quite arbitrarily assumed, without the least evidence that such is the case. Readers of my paper[2] will recall that definite evidence was there given to the contrary. Especial emphasis was laid on the fact that in the transparent living eggs of *Toxopneustes*, where the division of the cytasters can really be followed »Schritt für Schritt«, the asters are seen forming simul-

[1] Künstliche Parthenogenese, in Zool. Jahrb., Supplement, VII, Weismann Festschrift.

[2] Arch. für Entwicklungsmechanik, XII, 4 (vgl. S. 542, 578 usw.).

taneously in all parts of the egg and do not change their position until the first division-period, which coincides with the first nuclear division. This vital point is passed over by Petrunkewitsch without a word of comment and without bringing forward any corresponding observations on the living eggs. In point of fact, I figured conditions practically identical with all those of Petrunkewitsch's "series", except the remarkable ring-figure shown in his Fig. 19 (*e. g.* my Figs. 2 *c*, 5 *a*, 3 *a*—*c*, from life; Figs. 25—35 from sections); but continuous observations on the individual living eggs gave strong direct evidence that these do not represent successive stages in the division of a single primary center, but are varying individual conditions, that often appear side by side in the same lot of eggs at the same time.

The author's main assumption that the "series" constructed from his sections represents a succession of genetically connected stages is made without presentation of the least evidence of a division of the centrosomes at any period, or even of the asters; for such spindle-connections as are shown in some of the figures (*e. g.*, Figs. 16, 19, 20) will not be considered valid evidence of a preceding division by anyone familiar with the common phenomenon of secondary spindle-formation between centers originally separate—indeed it was this very difficulty that I found so hard to overcome in endeavoring to establish, in sections of the entire eggs, the division of the cytasters and of the primary nuclear centrosomes — and it is obviously impossible that a closed ring-figure such as that shown in Fig. 19 (stated at p. 43 to be »offenbar ein unvermeidliches Stadium«) could arise as a whole by persistence of the original spindle-connections; one, at the least, of the connections shown, must have been secondary.

It is important to note that the so-called "series" of division-stages of Petrunkewitsch occurs at a period when the asters are large and conspicuous, and at the corresponding period in the living eggs of *Toxopneustes* are very clearly visible. It is therefore inadmissible to suppose that the discrepancy between his results and my own is owing to a failure on my part to observe the division of the asters at a sufficiently early period. I admit that my observations on the entire eggs do not exclude the possibility of a rapid multiplication of the egg-center at a very early period, before the asters have become clearly visible; but this is evidently not the period on which the conclusions of Petrunkewitsch are based.

Indirect evidence of importance is given by the time-relations, to which Petrunkewitsch seems to have given little attention, but he given a few significant details. In general, in his experiments

the eggs were exposed to the salt-solutions for a very long period (3—5 hours or even longer). In one of the cases figured (Fig. 9), three asters have formed after 5 hours in KCl-solution. Another (Fig. 21, stated to be of the same stage as Fig. 17; cf. p. 41), is the "stage" with four asters, which under his interpretation must represent the product of two divisions of the primary centrosome. This "stage" is from an egg treated for $4^1/_2$ hours with Na Cl-solution.

From these cases the mathematical reader may readily estimate the length of time that would be required at this rate to produce such a stage as that shown in Fig. 20, where the egg is filled with »echte Strahlungen« and centrosomes. Now, in *Toxopneustes* the exposure to the salt-solution was rarely as long as two hours, and often less than one hour; yet in this short time the eggs frequently developed hundreds of asters, and scores of these, as shown in sections, contained perfectly distinct centrosomes. That so great a number could arise in so short a time by the division of a single primary center is a priori improbable, and utterly impossible if the observed rate of division observed at a later period is assumed to be even doubled or trebled in the early stages.

It must, I think, be evident from the foregoing on what doubtful evidence the conclusions of Petrunkewitsch regarding progressive division are based. It is indeed possible, as I pointed out (op. cit. p. 555), that a division of the cytasters may take place at the time the nuclear bipolar figure forms; but my evidence of this was of the same inconclusive character as that produced by Petrunkewitsch, and must be supplemented by renewed observations on the living eggs.

The second point of Petrunkewitsch, namely, his failure to observe the formation of asters and centrosomes in enucleated fragments, is not open to criticism; though there is a rather puzzling inconsistency between the statement at p. 37 that such fragments »bleiben unverändert« and the one on the preceding page that radiations do appear in the fragments, though only in rare cases and in small numbers. Accepting the latter statement as corresponding to the facts, it is not surprising that so few asters were observed in non-nucleated fragments, or that these showed no central bodies; for, in *Toxopneustes* too, the asters frequently fail to form, and as stated in my paper (p. 562) they vary greatly in development, often showing no central bodies. Against this stands the fact that the asters of the enucleated fragments not infrequently do show as characteristic and definite a radiation, and as clearly marked a central body as in the whole eggs. It is not admissible to suppose that these asters are "primary radiations", or are otherwise not comparable with "true asters";

for, like the latter, they show large clear centers, their division was actually observed in the living fragments, and more then one aster was often observed in sections of single fragments.

A possibility of error is undoubtedly introduced by the shaking of the eggs to pieces; for it may well be that this somewhat heroic treatment causes the escape of nuclear substance into the cytoplasm, in the form either of minute portions of chromatin (which might escape detection in the living eggs, or even in sections) or of achromatic constituents. The importance of avoiding this possibility by cutting the eggs singly with the scalpel and treating the individual fragments is obvious, and the experiment has in fact been performed by Petrunkewitsch with results that appear to have been on the whole negative. Against this negative result may, however, be placed the positive ones (still unpublished) obtained by Mr. N. Yatsu, to which, with his kind permission, I am able to refer. At my suggestion Mr. Yatsu undertook the same experiment in the summer of 1903 on the eggs of *Cerebratulus* (treated with $CaCl_2$-solution) and on the very transparent eggs of *Echinarachnius* ($MgCl_2$-treatment). In the first named form, after individual section and isolation of the fragments, six cases of aster-formation were obtained in the enucleated half upon treatment with the solution; in the second, two such cases (out of 18 operated). In all these cases the asters were as conspicuous as in entire eggs and developed large clear centers (characteristic of the "true asters"); in three of them two asters were observed in each fragment, though an actual division was not observed. It may be added that in some of these cases the nucleated half divided, showing that the egg-center had not been removed by the section; fixed and stained preparations of the *Echinarachnius* fragments (not however sections) demonstrated the absence of a nucleus. These observations, made with suitable sterilization precautions by a skilful experimenter, are, I believe trustworthy and will be more fully reported hereafter, Yatsu's work being still unfinished. As yet these fragments have not been sectioned to show the presence or absence of centrosomes; but the character of the asters was that of »echte Strahlungen«, and leaves no doubt that they were neither the "primary radiation" nor the small "artificial radiations" of the type described by myself and Petrunkewitsch.

It appears to me from the above that the work of Petrunkewitsch fails to overthrow or even to weaken the case for the independent new-formation of centrosomes in the entire eggs; and the negative result attained in the case of enucleated fragments can not be accepted as decisive against the positive ones of myself and Yatsu,

unless further investigation shall reveal a source of error in the latter. I willingly grant that a phenomenon so surprising and of such far-reaching significance as the new-formation of centrosomes, capable of division, in a non-nucleated mass of protoplasm — or the hardly less remarkable one of a multiple free-formation in an entire egg of centrosomes capable of subsequent division — is not to be unreservedly accepted without additional study of the most careful kind, and by different observers; but if my conclusions on these points are to be rejected, it must be on evidence more adequate than that brought forward by Dr. Petrunkewitsch.

Stazione Zoologica, Naples, May 28th, 1904.

3. Über Fledermäuse von São Paulo.

Von Adolf Pira, Assistent am Zootom. Inst. d. Univ. zu Stockholm.

(Mit 2 Figuren.)

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Das zootomische Institut der Universität zu Stockholm hat neuerdings durch Herrn Apotheker R. Krone in Ignape eine Sammlung Fledermäuse erhalten, welche mir von Prof. W. Lechе zur Bearbeitung überlassen worden ist. Da diese Sammlung eine Reihe interessanter, teilweise wenig bekannter Formen enthält, teile ich folgendes darüber mit. Sie sind bei Ignape im Staate São Paulo, Brasilien, gefangen worden. Alle sind in Spiritus konserviert.

Fam. Vespertilionidae.

Vesperugo hilarii 1 ♀.

Bisher nur dieses Exemplar gefunden.

Die Angabe Dobsons (Catalogue of Chiroptera, London 1878), daß der letzte Caudalwirbel ganz und die distale Hälfte des vorletzten Caudalwirbels frei ist, kann ich nicht bestätigen, denn bei diesem Exemplar von São Paulo, wie auch bei drei andern aus verschiedenen Lokalitäten, die ich zu untersuchen Gelegenheit hatte, ist nur der letzte Caudalwirbel frei.

Atalapha cinerea brasiliensis 1 ♂.

In der Sammlung kommt eine Art von der Gattung *Atalapha* vor, die am nächsten mit *Atalapha cinerea* und den Varietäten derselben zu vergleichen ist, in vielen Verhältnissen aber sich so wesentlich von dieser unterscheidet, daß sie einer speziellen Untersuchung wert ist. Ich teile hier eine Beschreibung des vorliegenden erwachsenen männlichen Individuums mit.