oratories, and snap judgments should not be taken if the first isolations fail.

The results of Dr. Blumenthal and his associates are the more interesting because they started out on their crown-gall studies a half dozen years ago, entirely sceptical as to its cancerous nature. Along with all other Germans, they regarded it as a granuloma and only as they studied its behavior more and more were they gradually forced to change their views. The following is one paragraph from the summary in their second paper which is best given in their own words:

> Aus diesen Untersuchungen ergiebt sich, dass es zum *erstenmal* gelungen ist aus menschlichen Krebsgeschwülsten Parasiten zu gewinnen und in Reinkultur zu züchten, mit denen wir experimentell an Tieren bösartige Geschwülste erzeugen konnten. Diese Geschwülste lassen sich in vielen Generationen fortzüchten. Sie zeigten in ihrem histologischen Bau namentlich bei Übertragungen Carcinom-häufiger Sarkomcharakter, wuchsen bis zur halben Grösse des Tieres heran und bildeten Metastasen, die fast Walnussgrösse erreichten. Sie gaben bei der 4. Übertragung 75% Ausbeute. Auch an Pflanzen lässt sich mit diesen Kulturen, ohne Zusatz irgend eines Reizmittels wie Kieselgur u. dgl., eine Tumorbildung hervorrufen, die in ihrer Ausdehnung in nichts der durch *Bacterien tumefaciens* erzeugten nachgab. Wir glauben, dass die neoplastischen Bacillenstämme, die wir fortgezüchtet haben, dem *B. tumefaciens* nahestehen und mit diesem eine Gruppe bilden, die man als neoplastische Gruppe bezeichnen kann (p. 407).

ERWIN F. SMITH,
President of the American Association for Cancer Research
BERLIN, MARCH 5, 1925

SPECIAL ARTICLES

NOTES ON THE DEVELOPMENT OF THE SEA-CUCUMBER, THYONE BRIAREUS[1]

WHILE staying at the Marine Biological Laboratory, Woods Hole, Mass., in the summer of 1921, I was fortunate enough to have an opportunity, from June 21 to 24, to obtain eggs of *Thyone briareus,* which were reared successfully for nearly three months during my sojourn there.

The present preliminary account is mostly confirmatory of what is known in other Echinoderms, such as starfishes and sea-urchins, but since our knowledge of the embryology of holothurians is still meager, I think it desirable to put it on record.

In the ovarian tube of *Thyone briareus* are found eggs which may roughly be classified into three stages. To the first (1) belong late oogonia or early oocytes with a comparatively large nucleus, in which the chromosomes arrange themselves in pairs and often show more or less distinct polar orientation. The second stage (2) is characterized by diversities in the size of the cell body, which fact shows that the oocytes are now rapidly growing. In the nucleus there are a number of nucleoli and the chromatin nets stain very faintly. In early ones, however, paired chromosomes are still recognizable. Among rather late ones the nuclear contents show homogeneous chromatic granulations, and the chromosomes, now gemini, are well formed and assume the shapes of O, X, Y, etc., scattered near the periphery of the nucleus. A single nucleolus is usually found. Probably this stage is passed very rapidly, and after that the nucleus again assumes the appearance of an ordinary germinal vesicle.

In the growing oocytes, but never in those of other stages, is found a peculiar structure in the cytosome. It consists of a few chromatic fibers in the form of a minute spindle or aster, sometimes surrounded by faint rays, and always situated near the end which later becomes the animal pole. It is found only in the specimens fixed with picro-sulphuric, but has invariably disappeared in those fixed with Bouin. A similar but somewhat different structure can also be seen in my slides of other sea-cucumbers, *Cucumaria saxicola* and *C. echinata.* Van der Stricht described a peculiar structure in the growing oocytes of the sea-urchins, *Echinus microtuberculatus* and *Sphaerechinus granularis,* and called it the astrophere. I believe it is identical with the structure in my preparations and should be taken as a modified centrosome (yolk-nucleus of some authors). The fact that it appears coincidently with the growth period suggests that the structure has something to do with the yolk formation.

The third stage (3), to which belong the full-grown oocytes ready to be laid, can be readily distinguished from the others by the presence between the egg surface and the follicular epithelium of a thick jelly coating. It is quite possible that between this and the preceding stage (2) a whole year intervenes, that is, the eggs of the second stage are to be laid next summer. The oocyte, now in its full size, is hemispherical or flattened sphere in shape, as is well known in the eggs of other sea-cucumbers. The germinal vesicle is large and lies eccentrically near the animal pole. This pole is usually directed toward the internal lumen of the ovarian tube, and is readily recognizable by the presence of a short conical process, which I once called the "micropyle appendage." The centrosomal structure, which was found in the preceding stage, is no more to be found. Instead of it, from the micropyle appendage a bundle of achromatic fibers

[1] Contributions from the Zoological Laboratory, Kyushu Imperial University, No. 1.

diverge toward the germinal vesicle. The wall of the vesicle nearest these fibers shows some wrinkles, among which is usually seen a chromatin mass of irregular contour. In some slides of *Cucumaria echinata* a number of chromosome threads radiate from this chromatin mass. This resembles very much the structure found in the spermatocytes of some insects and myriapods (Blackman, 1903; Meves, 1905; Payne, 1909; Browne, 1913; etc.) and which is known as the karyosphere. From comparison of these with the observations of Jordan and of Retzius on starfish eggs, I am inclined to think that in my case also the mass in question is the source of the chromosomes.

Often among the oocytes above described, one finds some irregular masses of large and small disintegrating yolk, inclosed in the germinal follicle like the oocytes. These are probably the eggs which had failed to be laid in the previous spawning season, and which have undergone degeneration. A remnant of the germinal vesicle may be found, unusually with enlarged nucleoli. Within the yolk masses a number of small nuclei can be found, and I think that they represent phagocytes. Similar features have already been noticed by Gerould in *Caudina arenata,* by Théel in *Mesothuria intestinalis,* and by Caullery and Siedlecki in *Echinocardium cordatum.*

Spawning always occurs late in the afternoon of the day in which the animals are brought into the laboratory. By putting the animals in dim light, however, I could induce them to spawn even in day time. When returned to light they suddenly ceased to spawn, but in dim light laying was again observed. Utter darkness, on the other hand, did not cause them to spawn. In no case could I observe isolated females spawning spontaneously, but it is always preceded by sperm emission of a male or by males lying near.

Observations of Newth on *Cucumaria normani* and of Ohshima on *C. echinata* agree in that the eggs when laid have already formed the second polar spindle. This seems to be incorrect so far as my new observations on the present species justify. The eggs pipetted immediately out of the genital papilla show invariably the *first* polar spindle in rather early metaphase. In no case have I found the nuclear membrane remaining. The germinal vesicle is represented by the so-called residual substance, near the center of which are seen one or several nucleoli diminished in size.

Judging from the fact that the germinal vesicle remains intact in early stages of the degenerating oocytes, as mentioned above, it seems probable that the breaking down of the germinal vesicle takes place after the egg becomes detached from the ovarian wall, but before it leaves the genital papilla, *i.e.,* while the egg travels through the oviduct.

The egg freshly laid measures 260 to 300 μ in the largest diameter across the equatorial plane, and 200 to 210 μ in thickness, *i.e.,* along the egg axis. The jelly membrane, now free from follicular epithelium, measures 60 to 80 μ in thickness and shows radial striations as is well known in other cases. The first polar body is pinched off within about 20 to 30 minutes after the egg is laid. As it is absolutely impossible to keep the eggs quite free from sperm, it is hard to say whether the polar division is stimulated by the presence of sperm or not, although it seems highly probable that the first polar spindle is arrested at the metaphase stage until the entrance of a sperm. In the eggs which have already formed the first polar body a sperm head could always be demonstrated in stained sections. The second polar body is constricted off from the egg about 30 minutes after the first. While forming the polar bodies, the egg changes its shape obviously from alternate changes in viscosity of the plasm. It becomes more rounded during divisions and again flattens after the throwing out of the polar bodies.

The chromosomes on both the first and second polar spindles do not divide synchronously, and they do not form any regular equatorial plate at the metaphase. It is therefore quite difficult to tell their exact number; the probable haploid number being 22.

The centrosome is usually invisible in the first polar spindle, but in the second it is easily seen on each end of the spindle. After the second polar division the chromosomes belonging to the egg are more or less regularly arranged in a plane and swell up to form each a chromosomal vesicle. In those lying peripherally the change takes place more rapidly than in the central ones. At the same time the group of growing vesicles move down, preceded by an aster, toward the center of the egg, and they rapidly fuse to form a single egg nucleus.

The entrance of the sperm takes place most frequently near the equator of the egg, but rarely at the vegetal pole, while no sperm whatever has been found to enter at or near the animal pole. At first it is of the shape of a chestnut, with the aster radiating from its apex, but it soon becomes an ovoid vesicle. Whether its centrosome divides during the migration I am unable to say, because, as a rule, the centrosomal structures are rather indistinct in this material. The two germ nuclei meet at the center of the egg, and as they are at that time of about the same size and structure, one can distinguish them only from their positions. The egg nucleus usually lies on the side of the animal pole, while the sperm nucleus is found either lateral or nearer the vegetal pole.

There seems to be a stage when the nuclear membrane between the apposed germ nuclei disappears, thus forming a single first cleavage nucleus. The first cleavage spindle soon appears, although the origin of its centrosomes could not be followed with certainty. Chromosomal vesicles are formed at the anaphase of the division. The cleavage furrow appears first at the animal pole and proceeds faster at this side than at the vegetal. The 2-cell stage is reached within about two hours after spawning, then after half an hour comes the 4-cell stage, and the 8-cell stage after still another half hour.

For the account of the further development no detailed study has as yet been made, and it must be postponed to future communications; a rough sketch of observations may, however, not be superfluous here.

As I have before noticed in *C. echinata,* the blastomeres arrange themselves in a spiral manner in the present species also. The spirality is not so regular as is seen in those typical cases, such as in Turbellaria, Polychaetes and Gasteropods.

A gastrula is formed by the 18th to the 20th hour. It has cilia all over the surface of body, but is still inclosed within the egg membrane, often with the polar bodies attached at one side. The so-called *dipleurula* stage also is passed within the egg membrane, and it is not until the *metadoliolaria* is fully formed, when three and a half days old, that the young holothurian becomes free from the egg membrane and creeps out. Thus a free-swimming stage is here entirely lacking. The only other known example of this sort is *Holothuria floridana* as studied by Edwards. The *metadoliolaria* just escaped from the egg membrane has a big hanging preoral hood, five unbranched tentacles and a pair of ventral pedicels.

The preoral hood is gradually absorbed and the animal can now be called the *pentactula.* It creeps about and feeds on detritus found on the bottom of the vessel. The tentacles begin to branch, and new ones appear until the normal number of ten is reached. Ventral pedicels also increase in number, and calcareous deposits, in the form of plates, tables and rods, appear on the walls of body, of tentacles and of pedicels.

In conclusion, the fact is noteworthy that, despite the small size of the egg, no planktonic larval stage appears in this species. On the other hand, some sea-cucumbers with large yolky eggs, such as *Cucumaria frondosa* (up to 650 μ, Nordgaard), *Psolus phantapus* (600 μ, Runnströms) and *Cucumaria echinata* (440 μ, Ohshima), produce typical free-swimming larvae.

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HETEROPLASTIC GRAFTS OF THE ANTERIOR LIMB-LEVEL OF THE CORD IN AMBLYSTOMA EMBRYOS

The following is a brief report on the results of some preliminary experiments carried out in the spring of 1924 for the purpose of testing the effect of a foreign source of innervation on the development of the anterior limb-bud of *Amblystoma* embryos. That the anterior limb-rudiment of *Amblystoma* is self-differentiating and, therefore, independent of nervous influence, at least in early stages, is indicated by the work of Harrison and his students. Harrison has replaced the limb-buds from *A. tigrinum* with those of *A. punctatum,* and vice versa, and found that the transplanted limb-rudiment developed very much as it does in its normal location. In the present experiments, the limb-level of the cord in these two species was exchanged, the limb-rudiments remaining in their normal positions. In carrying out the operations, two embryos, one of each species, were arranged side by side and a segment of the cord corresponding to somites 3, 4 and 5 was excised from each and reimplanted in normal orientation in opposite embryos. Sixty-six pairs were operated upon in this manner, the results of which are summarized in the following table:

Series	Operations	Embryos	Stages	Dead at end of seven days	Survivals
I	5	punct.	24	5	0
	5	tigr.	25	5	0
II	16	punct.	21	13	3
	16	tigr.	24	16	0
III	18	punct.	24	15	3
	18	tigr.	26	15	3
IV	12	punct.	23	10	2
	12	tigr.	25	12	0
V	15	punct.	23	15 (1 fixed)	0
	15	tigr.	25	15	0
Total	132			111	11