EXPERIMENTAL METAPLASIA

1. THE FORMATION OF COLUMNAR CILIATED EPITHELIUM FROM FIBROBLASTS IN PECTEN

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EIGHT FIGURES (THREE PLATES)

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INTRODUCTION AND REVIEW

The experiments described in this paper were performed on *Pecten maximus* and *Pecten opercularis* at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom.

In the course of some investigations as to the mode of formation of new fibrous tissue in *Pecten*, undertaken by Mr. W. De Morgan and myself (Drew and De Morgan '10) we transplanted various tissues, such as the digestive gland, gills, gonad, etc., into the middle of the adductor muscle, and studied the process of fibrous tissue formation around the implanted mass. We also injected sterile Agar jelly made with sea water, and since then I have performed similar experiments with sterilised cotton wool, cork, elder

pith and other foreign bodies which are presumably unaffected by the body fluids of Pecten. In these experiments it was noticed that the reaction of the tissues to the implantation of portions of the ovary presented, in the later stages, marked differences from the reaction to any of the other tissues or foreign bodies employed, and it was with the object of explaining these differences that the work here described was undertaken.

Briefly summarised, my results show that after implantation of a portion of the ripe ovary into the adductor muscle, a layer of fibroblasts is formed around it, and coincidentally the ovarian tissue is invaded by phagocytes and degenerates. After the lapse of about six days no trace of organised ovarian tissue remains, but there is left a cyst surrounded by fibroblasts, and containing blood corpuscles and a quantity of small granules having the orange color of the yolk substance. After the lapse of about 20 days more, the innermost fibroblasts gradually change their shape, and form a layer resembling columnar epithelial cells, which later become ciliated. Eventually the whole cyst becomes lined with well defined ciliated epithelium, which persists at least for 120 days, which is the longest period I have yet succeeded in keeping the animals alive in the experimental tanks. I consider that there is some evidence to show that this change of the fibroblasts into ciliated epithelium is a reaction to the presence of some definite chemical substance within the cyst.

In the vertebrate many cases of the change of a tissue of one type into another type are known to occur, and to such changes the name Metaplasia has been given. According to Adami, ('08) they have this in common, that "Epithelial (epiblastic and hypoblastic) tissues can only be converted into other forms of epithelial tissue, and one form of mesoblastic into another form of mesoblastic."

Such examples as the conversion of cartilage into bone, or of connective tissue cells into fat, are cases of physiological metaplasia of mesoblastic tissues, and it is to be noted that these changes occur in the adult and not only in the course of development. Instances of pathological metaplasia are numerous. Thus among the epithelial tissues we have the change of the columnar ciliated

epithelium of the larynx into a squamous type as the result of chronic irritation, the conversion of the pavement epithelium of the bladder into the columnar type found in papillomatous overgrowths, and a similar change in cases of ectopia vesicae where even glandular crypts of a very simple type may appear.

Of special interest are the experiments of Wolff ('93) on the eye of the larval newt and salamander. In these animals, if the lens be removed, it is regenerated from the iris, and it has also been shown that the retina itself can, under certain conditions, produce bodies resembling lenses. Here we have a case of metaplasia between totally dissimilar tissues, differing greatly in their mode of formation, but which still are both of epiblastic origin. A somewhat similar case is that described by Saxer ('04) who found a tissue resembling columnar epithelium lining the cysts that are of relatively common occurrence in gliomata.

Among the mesoblastic tissues there are many common instances of metaplasia. For example, the ossification of tendons and muscles, the formation of true bone in the lungs, in old pleural and pericardial adhesions, and in the fibroid valve of chronic endocarditis. Harvey ('07) has experimentally caused the formation of bone in the walls of arteries of rabbits by injury, and a similar formation is often found in cases of arterio-sclerosis of long standing.

Examples of the change of tissue derived from one germ layer into closely allied tissues derived from the same germ layer might be multiplied indefinitely, but it must be noted that there are many pathologists who deny this frequent occurrence of metaplasia, and explain these changes in other ways. Foremost of these is Ribbert. He considers, for example, that the replacement of columnar by squamous epithelium is due to the overgrowth of included islands of squamous cells, under altered conditions of environment, and he has other explanations for most of the cases here cited. His conclusions, as summarized by Adami ('08) are to the effect that "only tissues that, while externally different, possess nevertheless the same histogenetic capacities, can undergo metaplasia, one into the other." On the other hand, Leo Loeb ('99) has recorded that in cases of epithelial regeneration

in vertebrates, he has observed epithelial cells migrate into the underlying tissues, and take on the appearance of fibroblasts.

Much of the work that has been done on regeneration is of interest in this respect. Braun ('03) has pointed out that in regeneration in tadpoles, epithelium may give rise to nerve tissue. There is the work of Barfurth ('91-'00) and Fraisse ('85) on regeneration of the tail in several Urodela, and in the tadpole of the frog, and of Towle on the limbs of *Plethodon*. These observers find that each tissue only reproduces tissue similar to itself, but their experiments were necessarily confined to types in which regeneration occurs, and dealt only with the phenomena consequent on amputations. Much more work has been done on the regeneration of single tissues or organs, and in these cases it has been found that like always reproduces like, and that metaplasia does not occur.

To turn now to invertebrates, we have the observations of Miss Reed, recorded by Morgan, ('04) on the regeneration of the claw of the crayfish and hermit crab, in which it is conclusively shown that the whole claw with its muscles, etc., is regenerated from the ectoderm, *i.e.* metaplasia of ectodermal into mesodermal cells occurs. Again there are the experiments of Koeber ('00) on the regeneration of the pharynx of *Allolobophora*, in which he shows that though the epithelium lining the pharynx is developed from the ectoderm, yet in the course of regeneration it grows from the endoderm continuous with that lining the alimentary canal.

Flexner ('98) has pointed out that in regenerating Planarians the surface epithelium may give rise to distinct nerve elements, and in this case, though both the surface epithelium and the nerve tissue are originally derived from the same germ layer, yet we have metaplasia occurring between tissues which are histologically totally different, and indeed the process may in some ways be considered analogous to the regeneration of the lens from the iris in the eye of the larval newt and salamander described by Wolff ('94).

It thus appears that, whatever may be the case in vertebrates, in lower types of animals there is much evidence to show that metaplasia may occur between cells derived from different germ layers, and between cells possessing widely different histological

characters. With regard to the cytology of the changes involved in regeneration and metaplasia, Kölliker ('85) considered that regeneration of an organ or tissue cannot occur unless that organ or tissue contains cells of an embryonic character, or at least contains elements that are able to assume embryonic characters. The question has been more fully dealt with by Adami ('00), who comes to the conclusion that the fully differentiated cells of a tissue proper never arise from cells that are themselves fully differentiated, but during physiological regeneration arise from certain 'mother-cells' which are normally present in the tissues. He considers that "under abnormal conditions, the fully differentiated functioning cells of certain tissues are capable of proliferation and giving rise to cells of like nature, but this is only after a preliminary reversion to a simpler, more embryonic type."

It will be shown in the present paper that under the experimental conditions, the fibroblasts of *Pecten* revert to a somewhat undifferentiated embryonic type, and then become converted into columnar ciliated epithelium.

DESCRIPTION OF TISSUES INVOLVED IN THE EXPERIMENTS

An excellent and detailed account of the anatomy of *Pecten maximus* and *Pecten opercularis* is given by Dakin ('09) in his monograph on *Pecten*. The two species are very closely allied, and except in point of size, resemble each other both anatomically and physiologically to a remarkable degree.

The adductor muscle consists of two portions, bound together by the same sheath of connective tissue, but differing in structure. The larger, white and semi-transparent, consists of striated fibres. The fibres of the smaller, which is of an opaque and dead white appearance, and lies against the posterior surface of the larger mass, are non-striated. It was into the larger mass that all material in these experiments was introduced. Superficially the muscle is covered with a layer of columnar epithelium continuous with that lining the mantle.

There is a large blood supply to the muscle from the adductor artery (Dakin), and it contains numerous lacunar spaces. Scat-

tered through it are numerous strands of connective tissue. These contain fibroblasts with very elongated, deep staining nuclei, and long fibrillar processes.

The gonad consists of a semicrescentic mass attached at its base to the adductor muscle. When ripe the male proximal portion is creamy white in color, and the distal female part is of an orange or vermilion hue; the boundary between the male and female portions is sharply defined.

In *Pecten maximus* the loop of the intestine reaches almost to the apex of the ovary, whilst in *Pecten opecularis* it does not extend much beyond the testis.

Microscopically the gonad consists of branched tubules, terminating in alveoli lined with germinal epithelium: when ripe the alveoli are crowded and distended with ova or sperm, and it is not always easy to trace the connecting tubules. These tubules join up to form two main ducts which are lined by columnar ciliated epithelial cells, the height of which is about twice the width while the cilia are about as long as the cells (Dakin). Traced towards the alveoli, the cells lining these ducts become shorter and lose their cilia, and in the smallest tubules are of a flattened almost squamous type, where finally they appear as if they were directly continuous with the germinal epithelium.

The ripe ova are of an orange or vermilion color, measuring about 50 μ in diameter, the nucleus is relatively large, the nucleolus conspicuous, and the cytoplasm crowded with yolk granules. The spermatozoa are small and of the typical shape, with a long flagellum attached to the broad end.

The blood of *Pecten* is a slightly cloudy, colorless fluid, it does not coagulate, but when shaken a number of small, white, floccular masses appear, which soon fall to the bottom of the tube leaving the supernatant fluid clear and transparent. These masses consist of blood corpuscles, agglutinated to form plasmodia.

The corpuscles although varying in size, are only of one kind. They are amoeboid bodies, which when expanded protrude a number of slender pseudopodia. When contracted, they are ovoid or spherical. There is a single compact nucleus, staining

readily with methylene blue. The cytoplasm is finely granular, and stains with eosin, but there are no large eosinophil granules. According to Cuenot, ('91) they originate in a 'glande lymphatique' situated at the base of the gills.

In a former paper (Drew, '10) I have shown in the case of *Cardium norvegicum*, that when the corpuscles come in contact with a rough foreign body, or with injured tissue, they possess the power of agglutinating and forming a compact plasmodial mass. In this way bleeding from a small wound is stopped. When the edges of a wound are covered with this mass of agglutinated corpuscles, protoplasmic strands are formed across the wound, connecting the plasmodia, these strands thicken and contract, and so approximate the edges of the wound. I have repeated these observations on *Pecten*, and find that the same phenomena occur, and that a similar plasmodial mass of agglutinated corpuscles is rapidly formed around any tissue implanted into the adductor muscle.

That Lamellibranch blood corpuscles are capable of a phagocytic action towards degenerated cells has been shown by De Bruyne ('96) in the case of *Mytilus edulis*, *Ostrea edulis*, *Unio pictorum* and *Anodonta cygnea*. Sir Ray Lankester ('86 and '93) has shown that certain corpuscles of *Ostrea edulis* have a phagocytic action on diatoms and minute green algae, and I have shown (Drew, '10) that the corpuscles of *Cardium norvegicum* have a phagocytic action on bacteria, and are attracted towards extracts of dead tissues. It has also been shown that the corpuscles of *Pecten maximus* exercise a similar phagocytic action on dead cells (Drew and De Morgan '10).

The formation of fibrous tissue around a foreign body implanted into the adductor muscle has been described in a former paper (Drew and De Morgan '10). The normal fibroblast is an elongated cell, with a spindle shaped nucleus and an indefinite amount of cytoplasm which appears to be drawn out and connected with the neighboring cells by fine fibrillar processes. These cells are usually connected with each other by slender strands of some collagenous substance, which forms the groundwork of the fibrous tissue, and in the normal resting stage it is often impossible to dis-

tinguish between these collagenous strands and the fibrillar processes of the cytoplasm. When about to divide amitotically, the fibroblasts become shorter and thicker, the cytoplasm vanishes, and an oval or round nucleus with a reticulated arrangement of the chromatin results; eventually this splits in two and the two halves separate. The process is shown in fig. 1.

The details of fibrous tissue formation differ in the early stages according to the degree of irritative action of the foreign body, and the consequent amount of inflammation produced. If the inflammation is very slight, as was the case in most of the experiments about to be described, the implanted body is first surrounded by a thin layer of agglutinated blood corpuscles. This is followed by the rapid amitotic division of the fibroblasts in the neighborhood, they lose the typical spindle shape of their nuclei, and the new formed cells consist of rounded or oval nuclei with a scarcely perceptible amount of cytoplasm. These rounded cells migrate towards the implanted body, and arrange themselves in layers around it, the nuclei become elongated, and the proportion of cytoplasm increases. Finally a cyst wall of typical fibrous tissue is formed, surrounding and completely shutting off the implanted body.

METHODS

Both *Pecten maximus* and *Pecten opercularis* can be obtained in large numbers by dredging in the neighborhood of Plymouth. It was found necessary to allow these animals to become acclimatised to living in the laboratory tanks before proceeding to the experimental work. When first placed in the tanks, the mortality was heavy, often amounting to 30 per cent in the first three days, but after the lapse of about a week the survivors appeared to be fully acclimatised to the changed conditions, and often remained healthy for some months.

Experiments on animals whose health was doubtful were of no value, both because the shock consequent on the injection of the foreign body frequently caused death, and also because the reaction of the tissues was not normal in unhealthy specimens. When a *Pecten* is healthy, it lies with the valves of the shell

slightly apart, the tentacles are expanded, and it responds rapidly to any stimulus by closing the shell; when held up in the air the water which drains away is clear and contains no slime. An unhealthy specimen lies with the valves of the shell wide open, there is little or no response to stimuli, and the valves only close under pressure. The tentacles are retracted, and the gonads, gills, and tissues generally look flabby and unhealthy. The water which flows out between the valves is slimy and viscid, and this is generally the first sign of deterioration.

When making an implantation of the ovary, a healthy specimen was chosen in which the gonad was of a full orange or vermillion color, and obviously distended with ova. The valves of the shell were wedged apart with a cork, and the interior well washed with a brisk stream of sterile sea water from a wash bottle that had previously been steamed for some time in a 'Koch.' The adductor muscle was then severed with a scalpel, and one valve of the shell turned back. The extremity of the ovary was cut off, and while held by forceps, was very thoroughly washed with a stream of sterile sea water, then it was placed in a sterilised Petri dish containing a little sterile sea water, and kept carefully covered.

All instruments were sterilised by boiling in dilute caustic soda solution, and were washed in sterile sea water to remove any trace of the caustic soda immediately before use. They were always re-sterilised between each experiment. When not in use it was found more convenient to keep all steel instruments in lime water instead of drying them, as they are particularly liable to rust after being exposed to the action of sea water.

The transplanting needles, made of a platinum and iridium alloy, resemble large hypodermic needles. Two sizes were used, the larger for experiments on *P. maximus*, measured about 6 cm. in length, and 1 mm. in diameter, the smaller for experiments on *P. opercularis* was of the same length, but about half that diameter. Into the hollow needle a somewhat longer stilet fits closely, and works like a piston. Any material taken up in the point of the needle is sucked in by drawing the stilet back, and again ejected by pushing it forward. Small portions of the ovary

were cut off by fine scissors and drawn up into the needles in this way.

In the earlier experiments portions of the ovary were injected into the muscle through a hole bored in the shell. The holes were drilled in the convex or right valve by an ordinary dentist's drill, the head of which was prevented from penetrating too deep by a lapping of thread. The spot selected for drilling was sterilised with a saturated solution of corrosive sublimate, washed off with a solution of hydrogen peroxide (20 vols.) or distilled water, care being taken not to allow any of the sublimate to run between the valves. The transplanting needle was then introduced to the required depth, slightly withdrawn and its charge projected into the channel. The hole was then thoroughly dried, and stopped with sealing wax. In later experiments it was found that this proceeding, which occupies a good deal of time, was unnecessary, and the implantation was made directly into the muscle from the side. By this method there is a slightly greater risk of sepsis and the consequent death of the animal, but this is more than compensated for by the saving of time which it entails, and this is of importance when a large number of experiments have to be made. In some cases much larger pieces of the ovary were implanted by simply making a longitudinal slit in the muscle with a small scalpel, and inserting the ovarian tissue with fine pointed forceps. The wound thus made is closed by the contraction of the muscle, but the risk of sepsis when this method is employed is considerable, and only a small proportion of the animals survived the experiment long.

During the experiments the animals were kept in the laboratory tanks, or in basins with a continuous flow of water. Exposure to too strong a light should be avoided, and if the animals are required to live long it seems to be of advantage to cover the basins with green glass, or to moderate the light in some other way.

When required for examination, the animals were killed by wedging the valves of the shell apart with a cork, and then placing in dilute spirit. When dead, one valve of the shell was removed, and the adductor muscle carefully sliced with a razor until the orange color of the implanted ovarian tissue could be seen shining

through the semi-transparent muscle. A small cube of the muscle containing the ovarian tissue in the middle was cut out and placed for about three hours in Zenker's fluid, well washed, treated with dilute iodine, and finally embedded in paraffin. Serial sections were then cut, and stained in very dilute Delafield's hæmatoxylin. Other stains and fixatives were used for especial purposes, but the above procedure was found to be the most satisfactory as a routine method.

It is noteworthy that even after the ovarian tissue has been implanted into the muscle for as long as four months, the orange color is not lost or even diminished in intensity, so that the site of the implantation can always easily be distinguished.

RESULTS OF THE IMPLANTATION OF PIECES OF THE MATURE OVARY INTO THE ADDUCTOR MUSCLE, AND THE SUBSEQUENT DEVELOPMENT OF CILIATED EPITHELIUM FROM THE FIBROBLASTS FORMED AROUND IT

The sequence of events after the implantation of pieces of the ripe gonad of one specimen of *Pecten maximus* into the adductor muscle of other animals of the same species is identical with that occurring when the same experiments are performed on *Pecten opercularis*.

Pieces of the ripe ovary after ejection from the transplanting needle into the muscle, are roughly spherical in shape, and measure from 1 mm. to 0.5 mm. in diameter according to the size of the needle used. Very soon after implantation such a piece of ovarian tissue becomes surrounded by a thin layer of agglutinated blood corpuscles, and the track of the needle is closed by a similar mass of blood corpuscles forming a plasmodial mass, which, by its contraction, draws together the tissues that have been displaced by the passage of the needle (Drew, '10). This condition can be seen in sections from animals that have been killed about one hour after the implantation has been made. If the operation has been conducted aseptically, the resulting inflammatory reaction is very slight. There appears to be a definite determination of the blood cells towards the ovarian tissue, but there is nothing approaching

the condition of venous engorgement and stasis that occurs when a septic tissue is implanted (Drew and De Morgan '10).

After a time fresh blood corpuscles penetrate the thin agglutinated layer, and start a phagocytic action on the ovarian tissue. Meanwhile the fibroblasts in the walls of the blood spaces, and in the intermuscular connective tissue in the neighborhood, undergo division. This division is amitotic, and commences about twelve hours after the implantation. Before division the fibroblasts lose their spindle shape and become oval: a split then appears at one end, and progresses in the plane of the long axis of the nucleus until two daughter nuclei are formed, attached to each other at one extremity, and inclined at an acute angle to one another. These gradually straighten out until they form an hour glass shaped mass of nuclear material. Finally the two nuclei are separated at the constriction, and two oval or circular cells are produced, having large nuclei with relatively very little cytoplasm, and bearing no resemblance to the spindle shape of a resting fibroblast.

There follows a migration of these cells with round and oval nuclei towards the site of the implantation. They chiefly follow the course of the strands of fibrous tissue bounding the blood spaces, but many migrate in all directions between the muscular fibres.

On reaching the layer of agglutinated corpuscles surrounding the implanted tissue, the fibroblasts arrange themselves in rows; and their nuclei elongate in such a direction that their long axes form arcs of a circle surrounding the implanted ovary.

This surrounding layer presents a somewhat stratified appearance. At first it contains a number of blood-corpuscles, but these eventually are removed, probably by autolysis, leaving only the fibroblasts.

In these experiments the layer of fibrous tissue formed in this way was always very slight, usually not more than two or three cells thick. If by any error sepsis occurred, it was followed by a violent inflammatory reaction, and if the animal survived, by subsequent great formation of fibrous tissue.

Meanwhile the implanted ovarian tissue shows signs of degen-

eration. The cells of the germinal epithelium, the connective tissue cells, and the cells lining the oviduct, lose their normal appearance, the chromatin of the nuclei becomes aggregated into small darkly staining masses, and the outline of the cells becomes less distinct, the cilia of the oviducal epithelium soon vanish. Coincidentally the whole mass of tissue is invaded by blood corpuscles which exercise a phagocytic action and slowly remove the degenerated material. The mature, or nearly mature, ova show a much greater resistance to this degenerative process than any of the other cells.

Thus, after the lapse of about three days from the implantation, we have a mass of ovarian tissue which is invaded by phagocytes and shows signs of degeneration, and is surrounded by a layer of fibroblasts forming a definite cyst wall. The fibroblasts are mostly oval in shape, with little or no perceptible amount of cytoplasm, and have not taken on the appearance they present in the resting state.

From the fourth to the sixth day degeneration of the ovary continues, and when cyst formation takes place, as in the cases here described, the degeneration is complete and every trace of organised structure has vanished by the sixth day. When the degenerative changes are complete, the site of the implanted ovarian tissue is occupied only by blood cells, and by a granular substance, which must either be formed during the process of degeneration, or be left after all the other substances composing the ovarian tissue have been rendered soluble and so have escaped from the cyst. If the cyst is cut open, and the contents examined under the microscope, it is seen that the granular matter is of an orange color, and that many of the blood corpuscles have ingested particles of this substance. As the orange color of the cysts remains unchanged, and undiminished in intensity, even after four months, it appears that this substance is unable to escape from the cyst through the surrounding layer of fibrous tissue, and the same must hold true for blood corpuscles within the cyst, which have absorbed this substance by phagocytosis. It seems probable that such blood corpuscles, after ingesting granules of this substance,

degenerate, and eventually become dissolved, leaving the granules behind.

Fig. 2 shows the condition at the fifth day. The degeneration of the ovarian tissue is practically complete, though traces of the ova still remain: this tissue is surrounded by layers of oval fibroblasts, and the fibroblasts in the neighborhood are still dividing and migrating towards the cyst wall. Blood corpuscles are making their way into the cyst between the fibroblasts forming its wall.

The subsequent changes occur more slowly. The fibroblasts forming the innermost layer of the cyst wall remain unchanged for some time, but those forming the outer layers regain the typical elongated spindle shape of the resting fibroblast. Then, after a period varying from eighteen to twenty-five days from the implantation, the fibroblasts in immediate proximity to the degenerated ovarian tissue alter their appearance. The nuclei become rounder and the cytoplasm of each joins up with its neighbor, forming a faintly staining and somewhat indefinite continuous layer (fig. 3). Sections of a little later date show this layer more defined and also a change in the character of the nuclei. An aggregation of the chromatin resembling a nucleolus appears, and from this thin strands of chromatin radiate to the periphery. No cell walls are visible between the nuclei, which appear to have reverted to an embryonic type. Fig. 4 shows this condition, the contents of the cyst are surrounded by a continuous layer of nuclei with definite nucleoli, and these nuclei are embedded in a mass of cytoplasm having no dividing cell walls.

In the course of a few days these nuclei again alter in shape (fig. 5), they become smaller and more oval, and the nucleoli disappear. The surrounding cytoplasm becomes more definite, and stains deeper, and a distinct boundary or basement membrane appears between it and the layers of fibroblasts.

Shortly after this, long slender cilia appear from the inner border of this layer of cells (fig. 6). These cilia are very delicate and at first irregular in length, varying from a length about equivalent to the depth of the cells to more than twice that amount. The date of the appearance of cilia varied from 21 to 32 days, and seems to have some relation to the amount of ovarian tissue

implanted. Thus it certainly occurred earlier when large cysts, measuring about 4 mm. in diameter, were produced by cutting the muscle and inserting large pieces of the ovary, than when a transplanting needle was used.

In the course of time, lateral walls dividing the cells appear, and these are usually clearly visible about 40 days after the implantation. Thus eventually there is formed a closed cyst, the wall of which is lined with typical columnar ciliated epithelium and surrounds a mass of orange-colored granular debris, in which are numerous blood corpuscles in varying stages of degeneration (figs. 7 and 8). This cyst remains unaltered for at least 120 days, which is the longest period during which I have succeeded in keeping the animals alive under experimental conditions.

So far the sequence of events after implantation of pieces of the ovary producing subsequent cyst formation have been described, it will now be necessary to enter into the modifications of this process that occur when cyst formation does not take place.

If extremely small pieces of the ovary are implanted, or if, as sometimes happened in the experiments, the ovarian tissue was distributed thinly all along the track of the needle, cyst formation around the whole implanted mass does not occur. In such cases there is at first a very slight formation of a surrounding layer of agglutinated blood corpuscles, but beyond this, if the experiment has been carried out aseptically, there is very little reaction of the tissues to the implantation in the first few days. The implanted tissue often appears quite normal for as long as a week, or eight days, but after this, degenerative changes set in, and by the thirteenth day at latest, all trace of life in the cells of the oviduct, germinal epithelium, or ova has vanished. Meanwhile the degenerating tissue is invaded by blood corpuscles and fibroblasts. The latter tend especially to invade and travel along any remaining framework of connective tissue that may be left in the implanted mass, and thus to form a dense mass of new fibrous tissue in the place of the old. From the examination of a large number of sections of different stages of this process I am of the opinion that all implanted fibroblasts die, and that all the new fibrous tissue is derived from the cells of the host, though the matter would be extremely difficult to prove definitely.

As the actual disintegration of the ripe ova in the course of the degenerative changes occurs very slowly, the invasion of fibroblasts causes the ova to become completely surrounded by new fibrous tissue. Thus are produced a number of minute cysts containing very few ova, or possibly only one. Subsequently these cysts become lined with columnar ciliated epithelium, derived from the innermost layer of fibroblasts forming the cyst wall, in the manner already described.

It often happens, even when the whole implanted mass becomes encysted, that small aggregations of ova near the outside form separate cysts, and also develop a lining layer of ciliated epithelium. Similarly when small pieces of ovary are implanted, the fibroblasts, by traveling along any connective tissue framework present in the ovary, may divide the whole cyst into several partitions separated by thin layers of fibrous tissue, which subsequently also form ciliated epithelium.

EXPERIMENTS TO DETERMINE THE RELATION OF THE PRESENCE OF OVARIAN TISSUE TO THE FORMATION OF CILIATED EPITHELIUM FROM FIBROBLASTS

An attempt was made to cause the formation of ciliated epithelium by the implantation of portions of the ovary that had been killed in various ways, but all these were unsuccessful. The following methods were tried:

1. *Heat.* Small portions of the ovary were boiled in sea water for various times ranging from 2 minutes to 15 seconds, and then implanted in the muscle in the usual way. A considerable inflammatory reaction resulted, followed by extensive formation of fibrous tissue: a very large proportion of the animals died within a week of the implantations, and often the track of the needle did not heal up. In none of these experiments did the animals survive for more than 15 days, and in all these there was an opening leading to the surface from the implanted tissue.

Pieces of the ovary that had been heated for two minutes to 70°, 60°, 50°, and 45° C. before implantation gave similar results, but heating to 40° C. for two minutes in some cases did not affect

the ovarian tissue, that is to say, cysts lined with ciliated epithelium were produced in a small proportion of these experiments. The implantation of pieces of ovary that have been heated to 30° C. produces the same results as the implantation of unheated portions.

2. *Cold.* Small pieces of the ovary were frozen (at a temperature of -20° C.), and allowed to thaw slowly. The thawing process took about an hour, then after the lapse of 15 minutes the ovary was again frozen. This process was repeated four times, and then the pieces of ovary were implanted in the usual manner. The animals were killed after 35 days, and sections showed that a moderately intense inflammatory reaction had resulted; the implanted tissue was invaded and nearly completely replaced by blood cells, and was surrounded by a thick and compact layer of fibroblasts. No ciliated epithelium was present, nor did the inner layer of fibroblasts show any of the changes preliminary to its production.

3. *Chemicals.* Pieces of the ovary were treated for one hour with various protoplasmic poisons, then well washed in sterile sea water, and implanted. Solutions of the following substances were tried:

- 1 part corrosive sublimate in 2000 parts of sea water.
- 2 per cent phenol in sea water.
- 2 per cent of 30 per cent formalin in sea water.
- 1 part of 20 vols. hydrogen peroxide to 10 parts of sea water.
- 0.5 per cent of potassium cyanide in sea water.
- 20 per cent alcohol in sea water.
- 0.5 per cent of chloroform in sea water.

In every case the animals died within ten days of the experiment, showing signs of intense inflammation and often liquefaction of the tissues at the site of the implantation.

4. *Degeneration in vitro.* The ovary was thoroughly washed in sterile sea water, cut in small pieces, and each piece placed in a sterile test tube with a little sterile sea water. Similar pieces were placed in test tubes containing the blood of *Pecten*, collected under aseptic conditions. The ovarian tissue was allowed to degenerate in these tubes for three days, then cultures were made

with a loopful of the fluid from each tube on sloped fish broth peptone gelatin, made with sea water, and the pieces of the ovary were implanted into the muscle in the ordinary way. It was found, considering only those cases which the cultures showed to be sterile, that about the same proportion of the animals survived as in check experiments, but whether the degeneration had occurred in blood or in sea water, in no case was there any production of ciliated epithelium.

Experiments in which the ova were removed from the ovary and then injected were unsuccessful. If the ova be shaken out into sterile sea water, centrifugalised, or allowed to settle, and then injected with a hypodermic syringe, it was found that a large proportion of the animals died within a week, and in those that survived it was impossible to find the ova on dissection. To obviate this difficulty the centrifugalised ova were placed in a sterilised solution of gelatin in sea water, at a temperature just above the point of solidification, this was allowed to cool, and portions of the jelly implanted into the muscle: unfortunately this always resulted in the rapid death of the animal, presumably because the gelatin has some toxic action. Agar jelly could not be used for this purpose because it solidifies at too high a temperature, and also is not dissolved by the body fluids of *Pecten*.

A number of experiments were performed to prove that the development of the lining layer of ciliated epithelium of the cysts was not produced merely as a result of irritation, and as a reaction to the implantation of any foreign body. In a previous paper (Drew and De Morgan '10) the result of the implantation of the tissue forming the gills and digestive gland of *Pecten*, and of sterile Agar jelly, has been studied. In addition to these, such substances as sterilised cotton wool, cork, elder pith, and small portions of sterilised silicious sponges were implanted to act as a source of irritation. In all these cases the formation of a cyst wall composed of fibrous tissue took place, but there was no development of ciliated epithelium, and the same holds good for cases where the organ of Bojanus of *Pecten maximus* was implanted into other animals of the same species.

The transplantation of the ovarian tissue of animals of different

species into *Pecten maximus* or *Pecten opercularis* in every case caused death in a very short time. Such experiments were tried with the ovary of *Cardium edule*, *Cardium norvegicum*, *Glycimeris glycimeris*, and various species of *Tapes* and *Venus*. On the other hand, after the transplantation of the ovary of *Pecten opercularis* into the muscle of *Pecten maximus* and *vice versa*, the animals survived indefinitely, but there was no development of ciliated epithelium within the cysts formed round the ovarian tissue.

The implantation of portions of the male gonad produced a violent inflammatory reaction, with subsequent very extensive formation of fibrous tissue for some distance around the site of implantation. No cyst formation took place, and no trace of the spermatozoa could be seen in sections made three days after the experiment. The implantation of large pieces of the testis (more than about 1 mm. in diameter) usually caused the death of the animal in three or four days.

If small pieces of the ripe ovary be placed in sterile sea water containing a little sperm, then well washed and implanted, a similar inflammatory reaction and free formation of fibrous tissue results, only a small proportion of the animals survive, and in these no formation of ciliated epithelium occurred. Similarly, if some days after implantation of pieces of the ovary, a little sperm suspended in sterile sea water be injected with a hypodermic syringe into the exact site of the original implantation, inflammation and free fibrous tissue formation is set up, and the ovarian tissue is either absorbed, or becomes surrounded by a large area of dense fibrous tissue, and again the formation of ciliated epithelium is prevented.

With the object of eliminating the possibility of the formation of the ciliated epithelium lining the cysts from the epithelium covering the adductor muscle, which conceivably might have been invaginated by the introduction of the transplanting needle, a series of experiments was undertaken in which the implantation was made through a hole bored in the shell, and afterwards closed with sealing wax in the manner previously described. In these experiments the ciliated epithelial lining of the cysts developed in exactly the same way as when the implantation was made laterally through the muscle..

Another possible explanation of the development of the ciliated epithelium would be to consider that it was derived from the ciliated cells of the oviduct which ramifies through the ovary, though it would be difficult to understand how these cells could migrate to the walls of the cyst, and there form a continuous lining, while all the other parts of the ovarian tissue degenerate. To test this view two series of experiments were made. In one series only portions of the ovary taken from the extreme apex on the convex side were implanted: in this part of the ovary there is no oviduct, only the alveoli with the contained ova being present; the area thus free from the ciliated oviduct is small, but there is sufficient to make at least three implantations from each ovary with certainty that no ciliated cells are being introduced. In the parallel series of experiments portions of the ovary containing as much of the oviduct as possible were implanted. In both cases similar cysts lined with ciliated epithelium were produced. Other experiments were made in which pieces of the oviduct, which in its main branches is easily seen from the surface, were dissected out as carefully as possible, shaken in sterile sea water to remove any adhering ova, and then implanted: in these cases complete absorption and replacement by fibrous tissue often occurred, but in cases where cyst formation took place there was no formation of ciliated epithelium. Thus it is proved that the formation of this layer is independent of the presence of the ciliated cells of the oviduct.

Another point investigated was the relation of the ripeness of the ovary to the formation of the ciliated epithelium. It was found that this reaction did not take place as a result of the implantation of the spent or immature ovary, but only occurred when an ovary which was obviously full of ova, and of a bright orange or vermilion color, was used for the experiment. After the animals had been kept in the experimental tanks of the Laboratory for some time, the ovary often lost its bright color, and though full of ova became somewhat pale and unhealthy looking: experiments in which portions of the ovaries of such animals were implanted gave very uncertain results; sometimes the cysts were lined with ciliated epithelium, but more often this was absent,

and the cyst wall consisted only of fibrous tissue. If a thoroughly ripe and healthy looking ovary be used, and sepsis does not occur, it can be said with certainty that all surviving over thirty days will develop ciliated epithelium lining the cysts.

SUMMARY OF RESULTS OF EXPERIMENTAL WORK

The implantation of small pieces of the ripe ovary of *Pecten maximus* or *Pecten opercularis* into the adductor muscle of another animal of the same species results at first in the formation of a closed cyst within the muscle, lined with layers of fibroblasts. Complete degeneration and disintegration of the ovarian tissue within the cyst occurs in a few days, and then the cyst contains only an orange colored granular substance, presumably derived from the yolk, and numbers of blood corpuscles. After the lapse of from 21 to 32 days, changes occur in the innermost layer of fibroblasts lining the cyst, they revert to an embryonic type, and afterwards become converted into columnar ciliated epithelium, which forms a continuous layer lining the cyst. The changes resulting in this formation of ciliated epithelium from fibroblasts can be followed clearly step by step, and once formed, the ciliated cells persist unaltered for at least 120 days, which was the longest period for which the animals could be kept alive in the experimental tanks of the Laboratory.

Experiments were performed showing that this change is not produced by the implantation of any of the other tissues of *Pecten*, by neutral foreign bodies which would merely act as a source of mechanical irritation, by the transplantation of the ripe ovarian tissue of other Lamellibranchs, or by the transplantation of pieces of the ovary of *Pecten opercularis* into the adductor muscle of *Pecten maximus* and *vice versa*.

Other experiments showed that the development of ciliated epithelium does not occur if pieces of the immature or spent ovary be implanted, and that it is prevented by treating the ripe ovary with a suspension of the sperm in sterile sea water before implantation. Also that it does not occur if the ovary be killed by physical or chemical agents before implantation. A series of

experiments were made to eliminate the possibility of the origin of the ciliated epithelium lining the cysts from the ciliated cells of the oviduct, which might be present in pieces of the ovary that were implanted, or from the layer of epithelial cells forming the outer coating of the adductor muscle, which might be carried inwards by the transplanting needle.

It thus appears that the conversion of fibroblasts into ciliated epithelium is a specific reaction following the implantation of the ripe living ovary.

These observations are the result of nearly a thousand experiments, of which the majority have been performed on *Pecten opercularis*.

DISCUSSION OF RESULTS

It appears that the conversion into ciliated epithelium of the inner layer of fibroblasts lining a cyst formed round a piece of the ovary, which has been implanted into the adductor muscle of *Pecten*, is a specific reaction that occurs only when the ripe living ovary of an animal of the same species is implanted. The reaction takes place long after all trace of organised structure in the implanted tissue has disappeared, and it is difficult to conceive of its being due to any other cause than the presence of some definite chemical substance within the cyst, which is characteristic of, and specific for, each species. It cannot well be a fluid existing preformed in the ova, as in this case it would soon escape through the reticulate and easily permeable layer of fibroblasts first formed round the implanted mass, and for the same reason, considering the relatively great length of time necessary for the reaction to occur, it is not probable that it is a fluid formed at one definite stage in the process of degeneration. If a fluid at all, it seems on these grounds likely that this substance is slowly and continuously formed for a considerable period within the cyst. Examination of the contents of the cysts shows in all cases where the development of ciliated epithelium has occurred, that an orange granular substance, and blood corpuscles in various stages of

degeneration, are present. These orange granules resemble in appearance the orange colored yolk substance of the ripe ova, and the amount of this granular substance within the cysts seems to be independent of the length of time during which the implanted tissue has been allowed to remain in the muscle. If implantation of pieces of the ovary of approximately equal size have been made, examination of the contents of a cyst after 6 days will show as much of this substance present as in a similar cyst after 120 days, hence it appears that this substance cannot escape through the cyst wall. When it is considered that the development of the ciliated epithelial lining only occurs as a reaction to the implantation of ripe ova, containing a plentiful supply of the orange colored yolk substance, there is at least a possibility that the orange substance within the cysts bears a close relation to the yolk substance, and that the development of ciliated epithelium from the fibroblasts lining the cyst is a specific reaction to its presence.

Though based on no experimental evidence, I would suggest as a possible explanation of the phenomena, that some substance is formed as a result of the ingestion of these orange granules by the blood corpuscles, and their subsequent degeneration within the cyst: that the granules themselves remain unchanged, and are again set free on the disintegration of the corpuscles, and that their action on the protoplasm of the corpuscles is merely catalytic. This substance, produced from the blood corpuscles, is probably a fluid, and would be slowly and continuously formed as long as blood corpuscles could pass through the walls of the cyst. The action of this substance on the fibroblasts forming the walls of the cyst is to delay their return to the spindle shape typical of the resting condition, and eventually to set up those changes in the inner layer of fibroblasts resulting in their conversion of ciliated epithelium. Once this epithelial layer is complete, the access of fresh blood corpuscles to the interior of the cyst would be hindered or prevented, and so the formation of this substance would tend to stop; at the same time the outer layers of fibroblasts would regain the resting state, and form a layer of typical fibrous tissue which would tend still further to prevent the ingress of fresh blood corpuscles.

An obvious objection to this theory is that the ciliated epithelium is not produced after the implantation of pieces of ovary that have been killed in any of the ways described, but it must be taken into consideration that in these cases the implantation of the dead ovarian tissue was always accompanied by a more or less intense inflammatory reaction, and so the experiments are scarcely comparable to those in which portions of the living ovary were used. Also it is probable that the chemical composition of the ovary was altered by the methods of killing.

If by future experiments, and more extended observations, it can be fully substantiated that similar cytological changes take place in other types, as a reaction to the presence of some definite chemical substance, the fact should have an important bearing on the chemical theories of development, and possibly on the origin of certain abnormal heterotopic growths.

ABSTRACT OF EXPERIMENTS PERFORMED

Total number of implantations, including animals that died before examination (about)	950
Number of animals examined in studying stages up to 20 days	215
Number of animals in which cysts lined with ciliated epithelium were produced (not including those which died between 20 and 130 days, of which it is probable that most produced such cysts)	68
Experiments on effect of heat on ovary before implantation	42
Experiments on effect of cold on ovary before implantation	18
Experiments on effect of chemicals on ovary before implantation	48
Experiments on effect of degeneration on ovary before implantation	22
Experiments on implantation of centrifugalised ova	10
Experiments on implantation of ova in gelatin	10
Experiments on implantation of foreign bodies	33
Experiments on implantation of ovaries of other animals	46
Experiments on implantation of ovary of <i>P. maximus</i> to <i>P. opercularis</i>	12
Experiments on implantation of ovary of <i>P. opercularis</i> into <i>P. maximus</i>	12
Experiments on implantation of male gonad	20
Experiments on implantation of ovary first treated with sperm	14
Experiments on implantation of portions of ovary containing no oviduct	12
Experiments on implantation of portions of ovary containing oviduct	14
Experiments on implantation of unripe ovary	20

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PLATE 1

EXPLANATION OF FIGURES

- 1 Stages in division of a normal fibroblast. $\times 1000$.
- 2 Portion of a cyst wall after 5 days. Below is the degenerating ovarian tissue invaded by phagocytes, and this is divided from the muscle by several layers of fibroblasts, mostly in the active state. Above is a blood space bounded by fibrous tissue, some of the fibroblasts of which are dividing. $\times 800$.
- 3 Portion of a cyst wall after 20 days. The ovarian tissue has completely degenerated. The inner fibroblasts have changed the character of their nuclei and become connected by a continuous layer of cytoplasm. $\times 800$.

REFERENCE LETTERS

<i>b.c.</i> Blood corpuscles.	<i>div.fbl.</i> Dividing fibroblasts.
<i>b.sin.</i> Blood sinus.	<i>fbl.</i> Normal resting fibroblasts.
<i>cil.ep.</i> Ciliated epithelium.	<i>mig.fbl.</i> Migrating fibroblasts.
<i>deg.ov.</i> Degenerating ovarian tissue.	<i>mst.fbr.</i> Muscle fibres.
<i>mst.nuc.</i> Muscle nuclei.	

(N.B. The structure of the muscle tissue is merely indicated in the figures; details of striation, etc., are omitted.)

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PLATE 2

EXPLANATION OF FIGURES

4 Portion of a cyst wall after 23 days. The nuclei of the changed inner layer of fibroblasts have developed definite nucleoli, the cytoplasm is more definite than in fig. 3, and the cells are suggestive of an embryonic type. $\times 800$.

5 Portion of a cyst wall after 26 days. The layer of cells bounding the degenerated ovarian tissue has become well defined, with a distinct basement membrane; the character of the nuclei has again altered, they are smaller and the nucleoli have disappeared. $\times 800$.

6 Portion of a cyst wall after 30 days, showing development of long rather irregular cilia. The fibroblasts of the outer layers of the cyst are assuming the resting stage. $\times 800$.

7 Portion of a cyst wall after 98 days. The formation of ciliated epithelium is complete and dividing walls have appeared between the cells. The nuclei are smaller than in the stage represented in fig. 6. and the cilia are somewhat shorter and more regular. $\times 800$.

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PLATE 3

EXPLANATION OF FIGURES

8 Complete cyst lined with ciliated epithelium, of which fig. 7 represents a portion. The bands of fibrous tissue on each side of the cyst are formed in the track of the transplanting needle. $\times 400$.

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