CULTURES OF ORGANIZED TISSUES.

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PLATES 41 AND 42.

(Received for publication, May 31, 1922.)

It was shown by Thomson[1] that if a whole organ or a complete chick embryo 24 hours old was explanted to a culture medium, it increased markedly in size and no outgrowth of cells took place. If part of an organ, for instance a toe of a chick embryo, was explanted, an uncontrolled proliferation of cells began from the injured parts. When the basement membranes are injured, the cells begin growing out into the medium, whereas an uninjured organ continues to grow as a complete structure controlled by the laws of the organism. Thomson calls this "somatic growth." The increase of the organ stopped entirely after it reached a certain size. Possibly the cessation of growth was due to lack of absorption of the nutriment.

The experiments reported herein are in a way similar to those of Thomson. We used fragments of intestines from chick embryos which were about to hatch. At this period of embryonic development, the intestinal epithelium is assumed to possess its normal function.

I.

Technique.

The experiments were carried out in the following way. Chick embryos about 21 days old were taken out of the shell aseptically. The abdominal cavity was exposed and a fragment of the small intestine was extirpated and placed in Ringer solution. In some cases, the intestine was opened with a pair of fine scissors and a little strip was cut off and placed in the culture medium, which consisted of equal volumes of chick plasma and tissue juice. In other experi-

[1] Thomson, D., *Proc. Roy. Soc. Med.*, 1913–14, vii, Marcus Beck Lab. Rep., 71.

ments, the fragment of intestine was turned inside out, leaving the epithelium outside and the serosa inside the lumen. It was washed to free it from the meconial mucous secretions and transferred to the medium. After 48 hours incubation at 39°C., the culture chamber was opened and the fragment was picked out with the point of a cataract knife, or was aspirated in a pipette. Then it was washed for a minute or so, and transferred to a new medium. The small fragments of intestine brought about an extensive liquefaction of the clot, so that after 48 hours incubation, they were usually found floating in a cup-like excavation, surrounded with fluid.

II.

Description of the Cultures.

In some of the cultures, an outgrowth of connective tissue or epithelium from part of the fragment could be seen; in others, there was no uncontrolled growth whatsoever. After a few passages, the proliferation usually stopped and the epithelium could be seen growing all around the fragment, which was completely covered by epithelial cells after 48 hours. There was then no further uncontrolled growth. At this stage, the fragment had become spherical and its surface was as shiny as fresh mucous membrane. Under the microscope, it often appeared as a semitransparent body with a slightly denser central portion. In the early stage of life *in vitro*, numerous intestinal villi were visible and the individual cells of the cylindrical epithelium could easily be distinguished. Active peristaltic movements were observed around the free edge of the cuticula of epithelial cells. A broad, semicircular pseudopod was often observed to protrude from one cell, followed by a similar activity in the neighboring cells, whereupon the first one relaxed. It was also possible to perceive migration of the ameboid cells between the epithelial cells.

In many experiments, the active contraction of the intestinal muscles could be seen *in vitro*, even after a month's cultivation. These contractions appeared as slow as the peristaltic movement *in vivo* and were brought about by a slight cooling of the preparation.

The size of the spherical bodies, or "organisms," varied according to their age. In general, they tended to grow smaller as time went on.

When the tissue was transferred to a fresh medium, the body contracted markedly, due to cooling and irritation during the manipulation, but after incubation for an hour or two in the new medium, it relaxed and consequently increased in size. Shortly thereafter, liquefaction took place around the epithelium, which secreted a mucous fluid. In a few cases, the actual secretion could be seen to exude from the epithelial cells. At the same time, several ameboid cells could be seen wandering through the stomata of the epithelial coat. They usually deteriorated rapidly in the mucus which surrounded the fragment. In later states, the intestinal body became pellucid, and the coat of regular cubic epithelium could be studied thoroughly. Often it was possible to observe small appendicular cystic formations which appeared on the surface of the body within a few hours (Fig. 1).

III.

Histological Examination.

After a month's cultivation, the tissues were fixed in 2 per cent formol-Ringer solution, and sectioned in series. They were found in an excellent state of preservation. The cylindrical epithelium had grown all around the fragment (Fig. 2). The villi had disappeared and the surface of the body was very even and smooth. The epithelium in the deep Lieberkühn glands was well preserved in many cases, but did not seem to have any communication with the outside. The connective tissue cells had formed a stroma with uumerous fibrillæ. There were no evidences of uncontrolled growth, as the epithelial cells did not go beyond their natural border-line, and there was no intermixing of the different cells.

It was supposed that bodies such as this would be able to live in a fluid medium. Therefore, fragments of intestines were cultivated in the usual way in chick plasma and tissue juice until they were entirely coated with epithelium. Then they were placed on the bottom of hollow slides and 3 or 4 drops of tissue juice were added. At the end of about a month, they were fixed, sectioned, and stained. There was a marked difference between those cultivated in the fluid medium and those in the solid medium. In the fluid medium, the villi were perfectly preserved, and very little stroma was left. In the

center of the "organism" only a faint shadow of the stroma remained. Just under the lining epithelium which appeared to be normal, were a large number of epithelial cells in a more or less ameboid state. Under this layer, an empty space could be seen which had probably been filled with liquid, and finally toward the center of the body, a very thin, loose stroma with single connective tissue cells.

The cylindrical epithelium was very well preserved. Its free surface formed a continuous and solid cuticula. This contact between the epithelial cells was only perfect in their peripheral part. Between the external and the basal parts of the cells, free spaces could be seen, as if they had been formed under the pressure of a liquid within the body. The epithelial border of one which had been kept in a fluid medium for a long time is shown in Fig. 3.

IV.

DISCUSSION AND SUMMARY.

An artificial organism, if one may so term it, composed of a complex of tissues, was cultivated for a long period of time. Small fragments of intestine from chick embryos 20 to 21 days old were placed in a suitable medium. The epithelium proliferated and completely covered the fragment of intestine after 4 to 6 days. A small body was thus formed, round or oblong in shape, surrounded by cylindrical epithelium and containing epithelial, connective, and muscle tissues, endothelium, and ameboid cells. After a month's cultivation *in vitro*, no necrosis had occurred. Therefore, it may be assumed that, through the intestinal epithelium, the medium supplied the intestinal tissue with sufficient nourishment. No uncontrolled proliferation took place after the epithelium had surrounded the entire fragment.

The cultivation of complex tissues will facilitate the study of the interactions of the different cells under various conditions. In some experiments, pure cultures of epithelial cells were grafted into such an "organism" without difficulty. The growth of malignant cells could be studied in the same way. When the "organism" was placed in a fluid medium, the epithelium remained normal but the stroma disappeared. It seems that plasma played an important rôle in the maintenance of the tissues in their normal condition.

V.

CONCLUSIONS.

1. Fragments of small intestine from a 21 day old chick embryo, cultivated in plasma and tissue juice, became completely surrounded with cylindrical epithelium.

2. After a month's cultivation in plasma and tissue juice, the tissues composing the mass were normal. It would seem that the necessary food material was absorbed by the epithelium from the culture medium.

3. When these masses were cultivated in embryo juice without plasma, the intestinal villi remained normal, while the stroma, connective tissue cells, and their fibrillæ progressively disappeared, leaving an epithelial cyst.

EXPLANATION OF PLATES.

PLATE 41.

FIG. 1. Experiment 2107–24. Section of an intestinal "organism" cultivated in embryonic juice without plasma. The epithelium is normal. The stroma is very loose and the cells are sparsely represented. A cystic formation is seen at *A*. × 150.

FIG. 2. Experiment 1085–6. Section of an intestinal "organism" cultivated in plasma and embryonic juice for a month. × 200.

PLATE 42.

FIG. 3. Experiment 2107–22. Section of an intestinal "organism" cultivated in embryonic tissue juice for a month. The loose stroma and the cubic epithelium with the dilatated spaces between the individual cells may be seen. × 272.

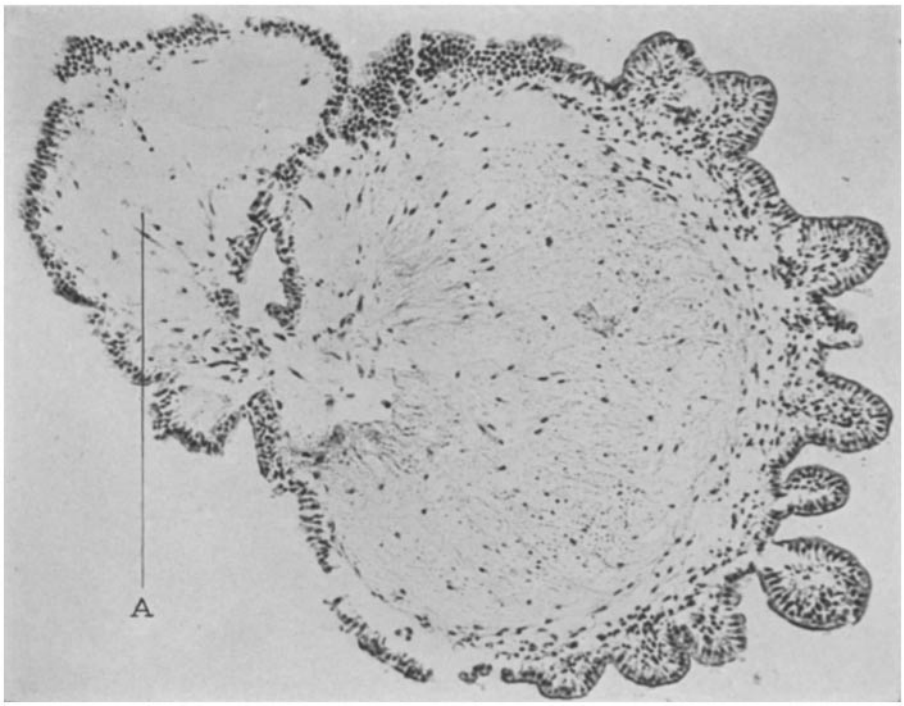

FIG. 1.

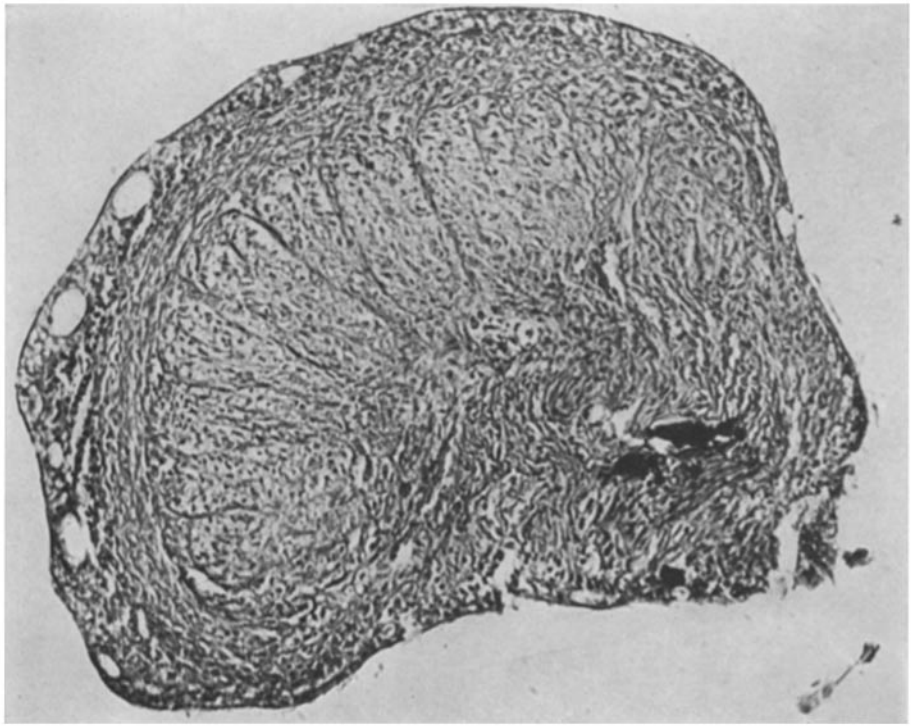

FIG. 2.

(Fischer: Cultures of organized tissues.)

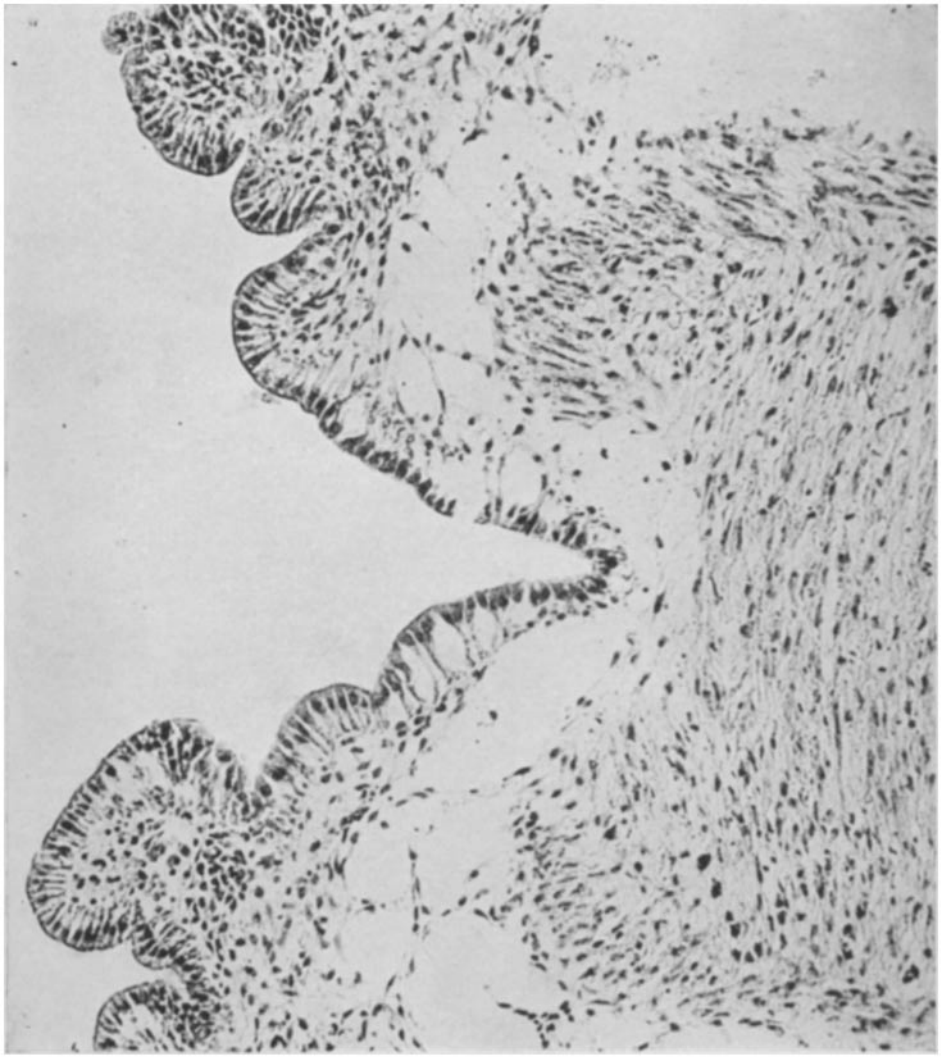

FIG. 3.

(Fischer: Cultures of organized tissues.)