II. LECITHAN COMPOUNDS.

Koch[1] has lately described the preparation of various compounds with lecithans, but it is uncertain whether these compounds are colloidal mixtures, mechanical mixtures or true chemical compounds. It seemed of interest to study the behavior of these substances in ether solution, when subjected to dialysis in rubber bags suspended in ether.

The preparations used in these experiments were made according to the method described by Koch. For the dialysis tests the solutions of the lecithan compounds were evaporated to dryness at 38° and the residues ground up with ether. The extracts were filtered and the filtrates placed inside of rubber bags and dialyzed against ether for thirty-seven days. The dialysates were tested every week to see if the substance combined with the lecithan diffused.

Compounds of lecithin with glucose, lactic acid, strychnin, digitonin, salicin, urea, creatin, creatinin and caffein were prepared. It was found that the glucose and lactic acid dialyzed completely, the strychnin, digitonin and salicin dialyzed partially, while urea, creatin, creatinin and caffein did not dialyze at all.

82 (607)

The relative importance of stroma and parenchyma in the growth of certain organs in culture media.

By **MOYER S. FLEISHER** and **LEO LOEB.**

[*From the Departments of Pathology of the Barnard Free Skin and Cancer Hospital and the City Hospital of St. Louis, Mo.*][2]

In experiments carried out about fifteen years ago, one of us observed that during the regeneration of skin, the epithelial cells are able to penetrate into and to grow in coagula of blood and of blood plasma.[3] This suggested to him that it might be

[1] Koch, *Journ. Pharm. and Exp. Ther.*, 1911, ii, p. 239.

[2] We wish to express our thanks to Dr. D. L. Harris, director of the Pathological Laboratory at the City Hospital, who put the facilities of his laboratory at our disposal at a time when our laboratory had not been finished; and also to Dr. M. G. Seelig, who very kindly assisted us in a number of our experiments.

[3] *Archiv f. Entwicklungsmechanik*, Bd. VI., 1898; *Johns Hopkins Hospital Bulletin*, January, 1898; *American Journal of Anatomy*, Vol. III., 1904.

possible to make various tissues grow in culture media outside of the body, in the thermostat, as well as inside the body, in the latter case the body acting as a thermostat. Inasmuch as he noted that the epithelial and also connective tissue cells grew preferably in contact with solid structures as fibres of fibrin, and into solid gelatinous material rather than into fluids he attributed stereotropic sensitiveness to various tissue cells, and he consequently employed more or less solid culture media as agar and coagulated blood serum for his various experiments. At first he carried out experiments in vitro as well as experiments in which the animal body acted as an incubator. Lack of the necessary facilities made it very soon necessary for him to limit himself to the latter kinds of experiments.[1]

To our knowledge in these our earlier experiments for the first time the attempt was recorded in the literature to grow tissues of higher animals under artificial conditions in environments that differ from those found in the body under natural conditions, to separate experimentally growing epithelial from connective tissue cells, and furthermore to study the influence of the addition of certain chemicals upon the growth of tissues.[2] Thus among other facts it was found that epithelial cells can grow in these gelatinous culture media even after addition of certain salts; that this growth can take place independently of connective tissue cells, that the epithelial cells may divide mitotically in the culture media, that they invade the coagulum through ameboid movements, that they have the power of phagocytosis, taking up into their body small particles of the culture medium;[3] that the growth ceases after this period of activity, and we explained on the basis of our observations certain phenomena of cancer growth as due to active ameboid ingrowth of cancer cells into the deeper tissues.[4] Later Harrison[5] showed in most interesting experiments that it is possible to grow embryonic nervous tissue of the frog in a mixture of fibrin and serum of the frog lymph, and recently Burrows

[1] Chicago, 1897; *Archiv f. Entwicklungsmechanik*, Bd. XIII., 1902. *The Journal of Medical Research*, Vol. VIII., 1902; *Journal Am. Med. Association*, 1901.

[2] *Zeitschrift f. Krebsforschung*, Bd. V., 1907, pp. 14 and 15.

[3] *Archiv f. Entwicklungsmechanik*, Bd. XIII., 1902.

[4] *Johns Hopkins Hospital Bulletin*, January, 1898.

[5] PROCEEDINGS SOC. EXP. BIOL. AND MED., Vol. III., 1907; *Journal Experimental Zool.*, Vol. IX., 1910.

extended in Harrison's laboratory the use of fibrin and blood serum as a culture medium to the growth of various mammalian tissues. Other investigators especially Carrel and his collaborators have within the last nine months taken up these studies and grown tissues in fibrin as well as in agar. We also resumed recently our former experiments and here we wish to report briefly a few of our results as far as they concern the relative importance of stroma and parenchyma in the tissue growth in gelatinous culture media:

A. After transplantation in vitro as well as in vivo, the central parts of stroma and parenchyma become necrotic, only the peripheral parts remaining alive. The extent of this central necrosis varies in different organs; it is, for instance, greater in the case of the kidney than in the case of the testicle.

B. In regard to the relative importance of stroma and parenchyma in the growth of certain organs in coagula we notice certain differences in various organs.

1. In the ovary of the guinea pig and rabbit either the whole piece becomes necrotic or in other cases the connective tissue and some follicles of the cortex remain entirely or partly alive. The epithelial covering of the ovary remained alive and even proliferated in a few cases. Mitoses in stroma or parenchyma cells or distinct ingrowth of connective tissue or epithelium into the coagulum were not observed. The greater part of the tissue became necrotic.

2. In the case of the testicle of the rabbit the peripheral alveoli usually remain alive without however any new formation of spermatozoa taking place. An irregular development of some epithelial cells into multinucleated giant cells may occasionally be observed. An outgrowth of the parenchyma into the coagulum does not occur, while the connective tissue can grow very actively into the coagulum.

3. In the case of the kidney an outgrowth of connective tissue into the coagulum was a very frequent occurrence especially in cases in which in the periphery of the transplanted piece a part of the connective tissue capsule had remained adherent to the parenchyma. The tubular epithelium in the periphery of the transplanted piece usually remains alive. Growth in the parenchyma takes principally place within the area of the transplanted

tissue, the regenerating cells of the tubules pushing the older cells into the lumen of the tubule and such desquamated necrotic cells glue together and form casts. Tubular epithelium can also grow between the transplanted piece of kindey and the coagulum and sometimes it penetrates into the coagulum forming occasionally canals in the latter. Mitoses are seen in the proliferating cells of parenchyma and stroma and such cells dividing mitotically may be seen lying directly in the coagulum. This description holds good for the ordinary manner of experimentation. Under certain conditions which we expect soon to describe in more detail it seems possible to increase very markedly the proliferating and infiltrating activity of the tubules.

4. In the case of the mammary carcinoma of the mouse the growth of the parencyhma is very much more prominent than in the case of the normal organs examined, a fact that is in accordance with the rapid growth of carcinoma cells in contact with animal cells in the body.

5. While various authors state that after the ordinary transplantation of carcinoma of the mouse into the subcutaneous tissue the stroma perishes and only the parenchyma remains alive, after transplantation into the culture media the stroma of mouse carcinoma remains alive and shows even certain growth phenomena. This observation should suggest a renewed investigation of the fate of the stroma after transplantation of carcinoma into the subcutaneous tissue of an animal.

6. Both parenchyma and stroma of the carcinoma of the mouse grow approximately equally well in coagulated blood plasma of the rat and of the rabbit.

7. The parenchyma cells of carcinoma penetrate very much more frequently into the coagulum than the parenchyma cells of other organs investigated so far. Mitoses are frequently seen in the carcinomatous cells and they may even be found in cells lying in the coagulum.

8. In regard to the skin we observed in our early experiments published elsewhwere the epithelial cells to penetrate in relatively large masses into the coagulum and we furthermore described at that time mitoses in these infiltrating epithelial cells.

C. We also noticed that the epithelial cells of the skin may

become phagocytic and take up small particles of agar and coagulated blood serum. Similar observations we made recently in the case of growing tubular cells of the kidney and of carcinomatous cells[1] and probably also in the case of ingrowing stroma cells.

D. While the parenchymatous cells penetrate onto the coagulum in a more or less definite arrangement which to some extent corresponds to the normal arrangement of the parenchyma cell, the form of cell columns or of tubules being retained respectively, the connective tissue cells on the other hand grow as single cells often sending out long drawn out stellate processes in the coagulum, the various connective tissue cells being loosely connected by such processes. This arrangement enables the stroma cells to penetrate into the coagulum with relatively greater ease than is possible in the case of parenchyma cells.

E. Stroma as well as parenchyma cells (including carcinoma cells) show a definite direction in which they grow into the coagulum, both having the tendency to proceed along the fibers which form in the coagulum and to follow, if at all, for a short distance only a course vertical to the direction of the fibers in the coagulum. The connective tissue cells seem however in consequence of their isolated and independent mode of growth and of moving occasionally to be able to penetrate into the coagulum in other directions more easily than parenchyma cells, but they also usually follow the road of least resistance.

F. We recognize therefore variations in the relative importance of stroma and parenchyma in the case of the growth of different organs. In a provisional way we may assume that the parenchyma of those organs or tissues that normally show an additive (expansive) growth (active outgrowth) like carcinoma and stratified epithelium of the skin show likewise an infiltrative growth in coagula (in gelatinous culture media generally) and that those organs that normally or during regeneration do not show additive (active expansive out) growth, but rather compensatory hypertrophy, do correspondingly not show a strong tendency to infiltrative growth, when growing in culture media (testicle, ovaries). In the latter class however the stroma may or may not show infiltrative

[1] The same observation has been recently made in the case of tumor cells by R. A. Lambert and F. M. Hanes, Journal Exp. Med., Vol. XIII, 1911, p. 495.

growth. Certain other organs like kidney apparently hold an intermediate position. Here both parenchyma and stroma may grow into the culture medium; the growth of the parenchyma is however under ordinary conditions relatively slight.

There are however in all probability other factors of importance besides the one just mentioned. Thus we observed so far in our experiments a very much more active growth of the connective tissue of the testicle than of the ovary. Perhaps the difference in texture of the organs is in this case one of the determining factors, the looser texture of the testicle being more favorable to the outgrowth of the stroma than the dense structure of the ovary.

We furthermore found differences and similarities in the manner of growth of stroma and parenchyma in the coagulum.

83 (608)

A possible significance of the Cammidge reaction.

By **L. B. STOOKEY.**

[*From the Physiological Laboratory, Medical School, University of Southern California.*]

Smolenski[1] attributes the Cammidge reaction to saccharose. This led us to think of some intestinal lesion as a possible source of the Cammidge reaction. Two possibilities seem to be evident (1) absorption of saccharose as such, (2) reversible action of intestinal saccharase.

To test this view the Cammidge test was made on urines in cases of "chronic intestinal disturbance." Twelve cases, only one of which showed a clinical suspicion of a pancreatitis, were studied. Five gave a positive Cammidge reaction. The case showing probably the most pronounced reaction failed to give the Cammidge test after 48 hours' starvation. During the twelve hours following the starvation period a liberal quantity of milk sweetened with levulose was given. This did not lead to a positive Cammidge.

From the experiments made thus far it seems probable that in cases showing a positive Cammidge there may be some relationship between the amount of cane sugar ingested and the intensity of the Cammidge reaction.

[1] *Zeitschrift für physiol. chem.*, 51, p. 127.