ON THE GROWTH OF EPITHELIUM

IN AGAR AND BLOODSERUM IN THE LIVING BODY

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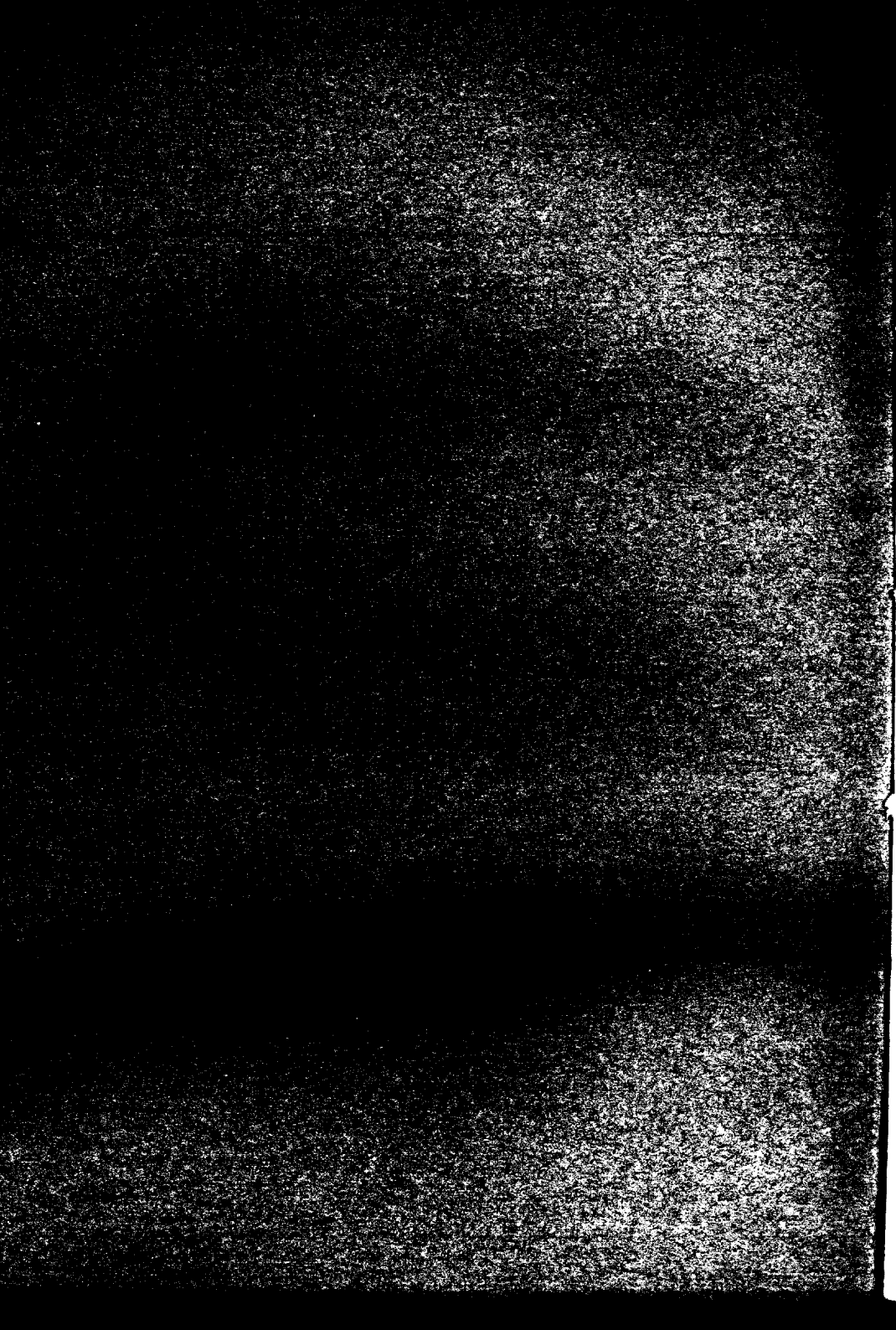
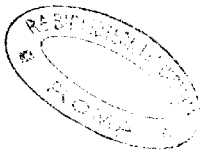




ON THE GROWTH OF EPITHELIUM IN AGAR AND BLOOD-SERUM IN THE LIVING BODY.[1]

LEO LOEB.

In a former paper[2] I described the method by which I aimed to find means, first, to observe the growth of different tissues like epithelium separate from other growing tissues; secondly, to subject regenerating, isolated tissues to changes in the chemical composition of the surrounding medium and to observe the influence of this change of conditions on the growth. I also gave some results of these experiments so far as they were connected with the problem of regeneration. I now want to show that in epithelium growing in bloodserum in a guinea-pig certain features are produced of interest to the interpretation of some structural peculiarities found in carcinoma. Above all, it must be stated in this connection that epithelium under these experimental conditions shows only a limited growth, and that it never develops a carcinoma. It is not the tension of the surrounding epithelium which prevents the epithelial cells from multiplying rapidly and from growing apparently indefinitely, as it occurs in carcinoma. It is not the connective tissue beneath which resists the indefinite growth of the epithelium. For this growth to take place, some special chemical or physico-chemical conditions must be present. Under such experimental conditions as given here, however, irregularities of growth appear which bear some analogy to the growth of epithelial tissues in malignant tumors. Epithelium growing in bloodserum usually does not produce keratohyalin, for the production of which a close union with the connective tissues seems to be essential. This change in the structure is entirely due to the

[1] Demonstrated March 28, 1902, at the Second Annual Meeting of the American Association of Pathologists and Bacteriologists, in Cleveland, Ohio.

[2] On the Growth of Epithelium. The Journal of the Amer. Medical Association, Oct. 19, 1901. A fuller report appeared in the Archiv. f. Entwickelungsmechanik, Vol. 13, where a detailed account of the method used is given.

conditions under which the growth is taking place. It is quite likely that all those changes in growing tumor cells, which have been interpreted as being essential conditions for the tumor growth (anaplasia of tumor cells) are only secondary changes produced by the abnormal conditions under which the growth is taking place.

The large number of epithelial pearls is a characteristic feature of many carcinomata derived from the skin. In epithelium growing in bloodserum, epithelial pearls are not rarely found. It was possible to determine in a number of cases, as the cause of these formations, the presence of foreign bodies; in this instance, of pieces of bloodserum. At an early period we see well-developed epithelial cells surrounding pieces of bloodserum. Figure 1 shows two such pieces encircled by well-developed, not degenerated epithelial cells. These latter are derived from pigmented epithelium, and therefore carry pigment themselves. This figure represents a relatively early stage, seven days five hours after transplantation of the epithelium into the bloodserum (Loeffler's bloodserum was used). Figure 2 shows a later stage of the same process, twelve days after the operation. The piece of bloodserum in the center is surrounded by several layers of epithelial cells, which are already somewhat advanced in the process of keratinisation. These pearls are not as large as the pearls usually seen in carcinoma, but this difference is probably caused by the different rate of growth in the ordinary and in carcinomatous epithelium. Not all epithelial pearls are caused by the presence of foreign bodies, but the presence of foreign bodies is one of the causes which is liable to produce them. In a former paper I have already pointed out that probably active movements of the epithelium are at work under these conditions. Figure 3 shows a small pearl formed without bloodserum. In its center we find an epithelial cell with a nucleus. The latter is surrounded by protoplasm. The outer part of the cell consists of fibrils arranged in a radiating way. This zone is surrounded by a well-defined membrane. This figure demonstrates one of the changes which may take

place in epithelial cells under these experimental conditions. Similar cells are not infrequently seen in carcinoma. The following figures also are reproduced to show the changes which are taking place in the epithelium under these conditions, and which when occurring in carcinoma have occasionally found a different interpretation. A cell differing from ordinary epithelial cells is represented by Figure 4. It shows an isolated epithelial cell which in the microscopic specimen, however, was surrounded by other epithelial cells. The nucleus shows here a peripheral hyperchromatosis. The chromatin granules, which are all in contact with the nuclear membrane, are connected with each other by threads. These chromatin particles probably were derived from the chromatin of the epithelial cell. As a remote possibility it must be conceded, however, that these chromatin granules were formed by immigrating leucocytes. Such a nucleus is not usually seen in an epithelial cell. This cell was found twelve days after transplantation. In epithelium growing in bloodserum not infrequently cells are seen like the isolated cell in Figure 5. This specimen is from a piece taken from the animal ten days after operation. The space between the isolated cell and the row of epithelial cells near by was probably originally filled by other epithelial cells, which, however, had disappeared. Another fact of interest is that epithelial cells transplanted into bloodserum often take up particles of bloodserum and include them in vacuoles. The nucleus of the cell usually surrounds one side of the vacuole. This fact I have already briefly mentioned in my former paper. The earliest stage in which I found this process to take place is shown in Figures 6 and 7, five and one-half days after transplantation of epithelium into bloodserum. In Figure 6 a large number of cells include pieces of bloodserum. These particles are of different sizes. Most of these cells carry much pigment, as is the rule for pigmented epithelial cells at this period of regeneration.[1]

The cells are not yet degenerated, as is especially well seen

[1] L. Loeb. Uber Transplantation von weisser Haut, etc. Archiv. f. Entwickelungsmechanik, Vol. 6, 1897.

in Figure 7, taken from the same piece. Here only a few cells contain bloodserum. Figure 8 shows particles of bloodserum in epithelium ten days after transplantation. The epithelium is quite vacuolar and many epithelial fibers are present in the periphery of the cells. In the vacuolar cells particles of bloodserum of different sizes are situated. It might be suggested that here perhaps a process of intracellular digestion has been taking place, and that the vacuoles were caused by particles of bloodserum which originally were included in these vacuoles. Figure 5 of the same period after transplantation shows bloodserum in a vacuole inside of the isolated cell mentioned before. Figures 9 and 10 show the presence of bloodserum inside the cells at a still later period, twelve days after transplantation. The cells are beginning to form keratinlike fibers. Their center frequently still contains protoplasm and a nucleus; the periphery, however, is often separated from the central part by a clear space. Several cells contain pieces of bloodserum in a sharply defined vacuole. At this stage of regeneration the pigment has disappeared from most cells. By what mechanism does the bloodserum get inside the cells? At early stages we can often see the epithelial cells surrounding particles of bloodserum. In the next stage the bloodserum is inside the cells. Figure 7 (five and one-half days after transplantation) and Figure 11 (twelve days after transplantation) show the epithelium penetrating into the bloodserum.

In Figure 7 no or very few leucocytes were present. In Figure 11 many leucocytes were present, and possibly opened a way for the epithelial cells. It is very probable that both these processes, the penetrating into the bloodserum and the taking up of small parts of it, are produced by slow protoplasmic movements of the epithelial cells. The particles of bloodserum are certainly often so large that leucocytes cannot have carried them into the epithelial cells. Lately Coates [1] and Ricketts [2] observed in blastomycetic dermatitis, blastomycetes as the center of the epithelial pearls

[1] Coates, W. E. A case of Blastomycetic Dermatitis. Medicine, Feb., 1900.
[2] Ricketts. Oidiomycosis of the Skin. Journal of Medical Research, Vol. 6.

(Coates), and blastomycetes inside of epithelial cells (Ricketts). In this case an active growth of blastomycetes into the pearls or cells can, of course, not be absolutely excluded.

A somewhat unusual occurrence is reproduced in Figure 12, where a piece of bloodserum twelve days after transplantation is surrounded by a cell very much resembling the chromatophores found in normal pigmented epithelium.

We have shown that, experimentally, a number of changes can be produced in the regenerating epithelium, which are also found in carcinomatous epithelium. These changes consist in (1) the formation of pearls; (2) certain variations in the structure of epithelial cells; (3) the production of cell inclusions. Two of these changes (Nos. 1 and 3) are directly determined by the presence of foreign bodies. The second change is only indirectly caused by the presence of bloodserum. These experiments may aid us in the interpretation of the structures found in carcinoma. They prove that certain structures are caused by processes which take place in certain epithelial cells alone; that other changes may be produced by the presence of substances foreign to the epithelium. This result suggests the possibility that some of the so-called cell inclusions in carcinoma may be produced in a similar way.

A series of experiments in which pieces of epithelium in bloodserum were removed at different periods of their growth for microscopic examination demonstrated that the changes taking place in the epithelium present a certain regularity with regard to the time at which they are found. Bloodserum is found inside the epithelial cells five days after transplantation. At this period epithelial cells are found at different places in the interstices of the bloodserum. These processes are further advanced at seven days. The cells, however, are still well preserved at this time. From now on more pronounced changes in the epithelial cells begin to take place which are quite apparent after ten days and are very distinct twelve days after the operation. Further experiments will have to be undertaken to determine whether by

certain variations of the methods employed a prolonged growth of the epithelium in bloodserum can be obtained.

Summary.

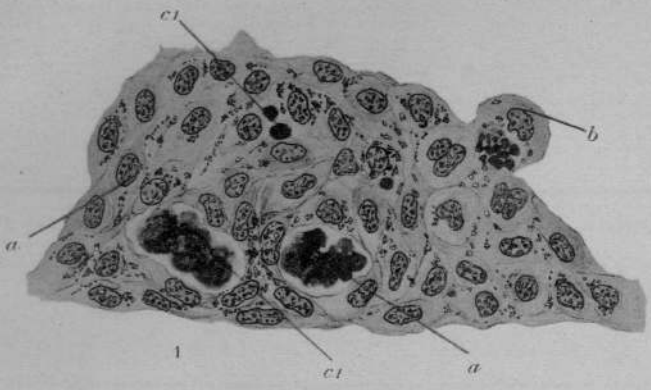
First: By transplanting epithelium into agar or bloodserum in the living animal changes can be observed resembling changes taking place in carcinomatous epithelium. Of the structural peculiarities thus produced, some are caused by degenerative processes in the epithelial cells, others by inclusion of foreign particles in the cells, still others by foreign bodies determining the arrangement of the epithelial cells (Formation of Epithelial Pearls). These experimental methods will aid us in determining what changes in carcinoma are produced by degeneration of carcinoma cells and what changes may be produced by the presence of foreign bodies.

Second: The inclusion of bloodserum in epithelial cells can be observed as early as five days after operation and can still be seen twelve days after operation.

Third: Pigmented epithelial cells transplanted into bloodserum show in the first twelve days the same changes in pigmentation which were found to take place in epithelium when it is regenerated in the usual way, together with connective tissue.

Fourth: The more pronounced degenerative changes in epithelium transplanted into bloodserum begin to take place about the eighth day after transplantation.

Fifth: These experiments prove that the changes found in carcinoma (a part of which may also be found in blastomycetic dermatitis) are not, so far as they have been referred to here, caused by any *specific* influence (for instance, blastomycetes), but are determined by the character of the epithelial cells, under the influence of chemically indifferent foreign substances.



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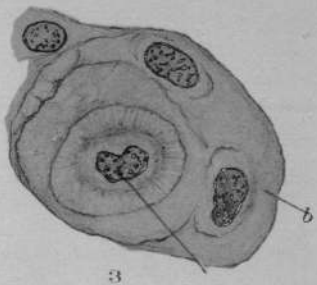

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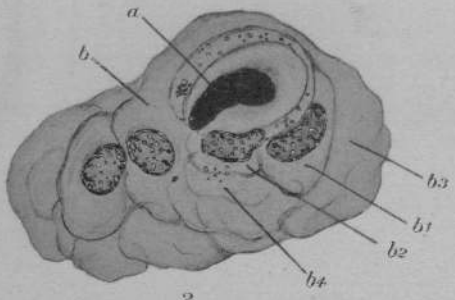

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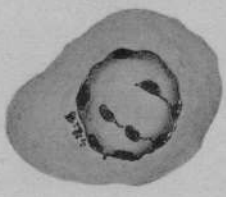

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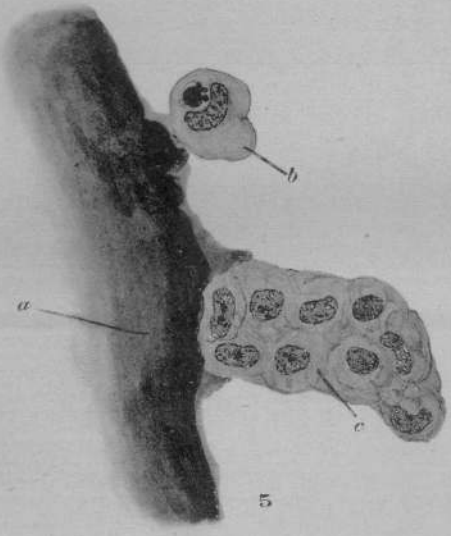

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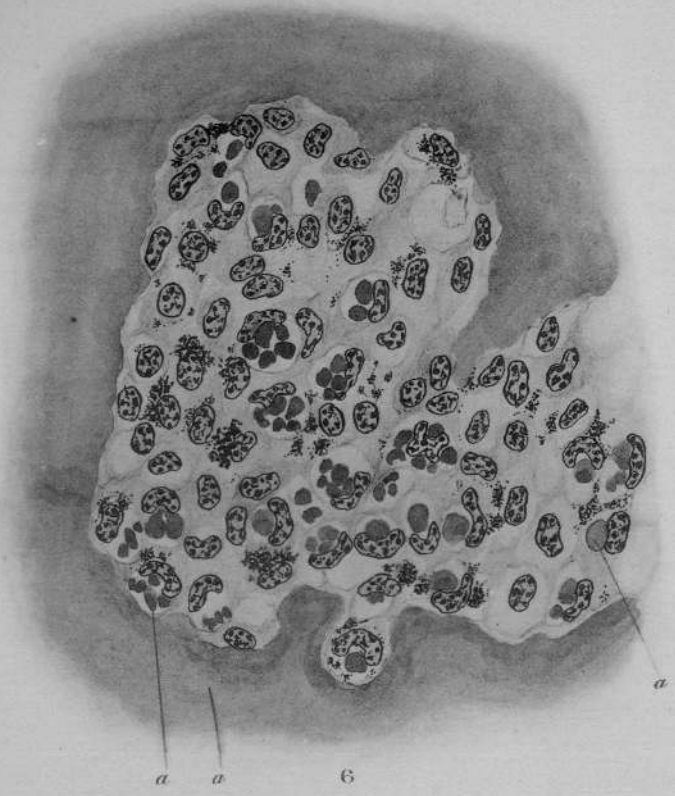

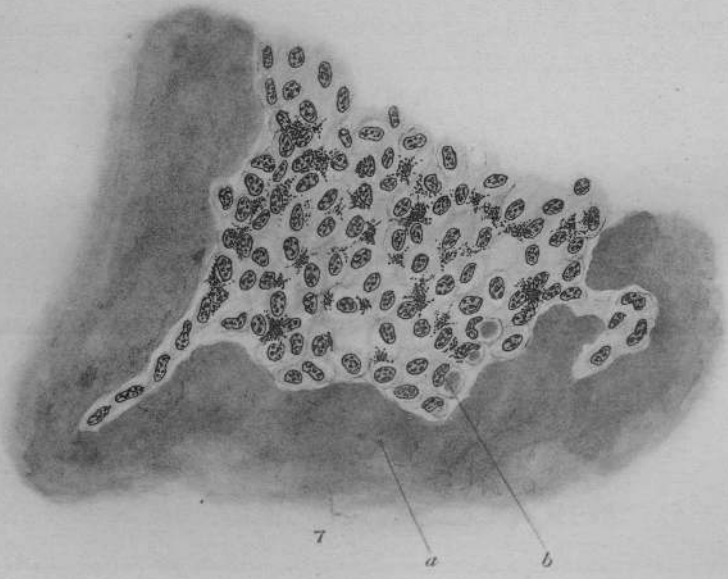

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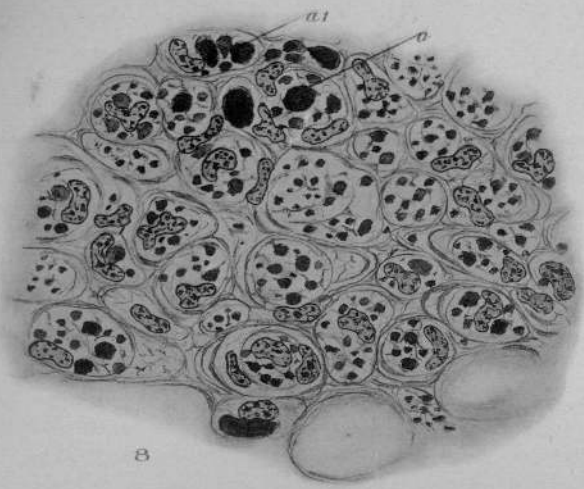

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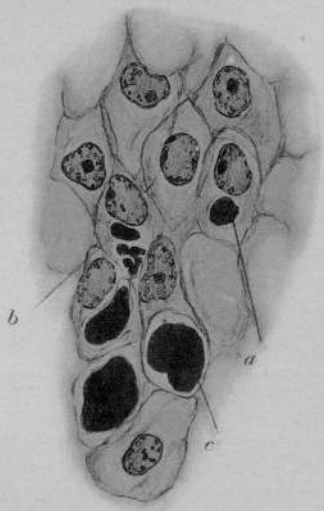

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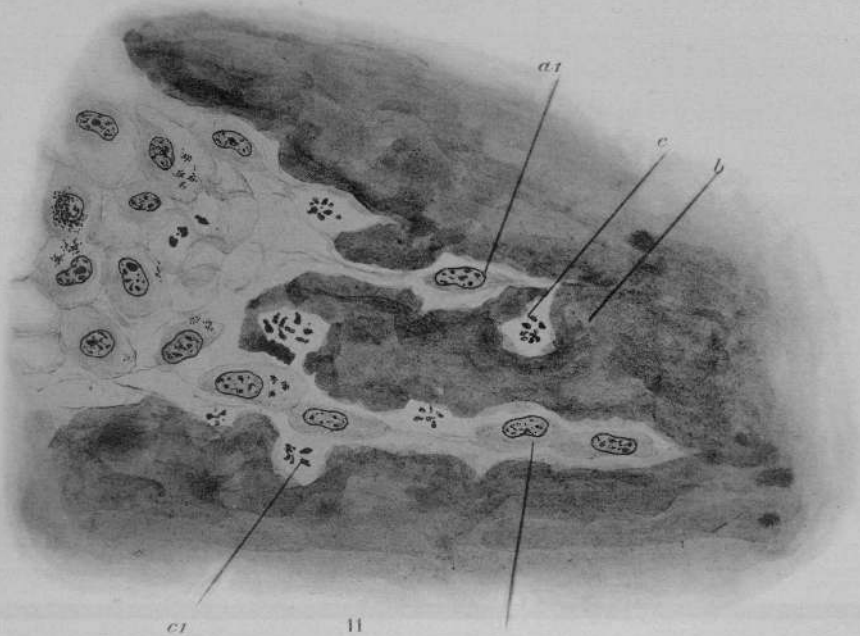

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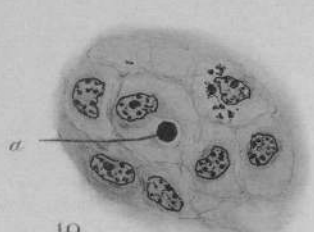

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L. Loeb.

Epithelial growth.

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DESCRIPTION OF PLATES.

PLATE IX.

FIG. 1. Epithelium, seven days and five hours after transplantation into bloodserum. a. Pigmented epithelial cells. b. Pigmented epithelial cell including bloodserum. c, c_1, c_2. Bloodserum between epithelial cells. Obj. $\frac{1}{12}$ x Oc. 4.

FIG. 2. Twelve days after transplantation into bloodserum. a. Piece of bloodserum. b, b_1, b_2, b_3, b_4. Epithelial cells around bloodserum. Obj. $\frac{1}{12}$ x Oc. 8.

FIG. 3. From the same piece as Fig. II. a. Striated epithelial cell, surrounded by epithelial cells b. Obj. $\frac{1}{12}$ x Oc. 8.

FIG. 4. Twelve days after transplantation into bloodserum. Epithelial cell with unusual arrangement of the chromatin. Obj. $\frac{1}{12}$ x Oc. 6.

FIG. 5. Ten days after transplantation into bloodserum. a. Bloodserum. b. Epithelial cell with bloodserum. c. Column of epithelial cells. Obj. $\frac{1}{12}$ x Oc. 4.

PLATE X.

FIG. 6. Epithelium, five and one-half days after transplantation into bloodserum. a. Bloodserum. Most of the epithelial cells include bloodserum. Many of the epithelial cells are pigmented, being derived from black epithelium. Obj. $\frac{1}{12}$ x Oc. 4.

FIG. 7. From the same piece as Fig. VI. a. Bloodserum. Epithelial cells with bloodserum. The majority of the epithelial cells are without bloodserum. Obj. 4mm. x Oc. 4.

PLATE XI.

FIG. 8. Bloodserum in vacuolar epithelial cells, ten days after transplantation. a, aa. Particles of bloodserum. Obj. $\frac{1}{12}$ x Oc. 4.

FIG. 9. From the same piece as Figs. II. and III. Bloodserum in epithelial cells, twelve days after transplantation. a, b, c. Bloodserum in epithelial cells. Obj. $\frac{1}{12}$ x Oc. 4.

FIG. 10. From the same piece. a. Particle of bloodserum in epithelial cell. Obj. $\frac{1}{12}$ x Oc. 4.

FIG. 11. From the same piece. a, a_1. Epithelial cells penetrating into blood serum. b. Bloodserum. c, c_1. Leucocytes. Obj. $\frac{1}{12}$ x Oc. 4.

FIG. 12. From the same piece. a. Cell resembling an epithelial chromatophor surrounding a piece of bloodserum, b. Obj. $\frac{1}{12}$ x Oc. 4.

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