CYTOLOGIC STUDIES WITH THE PHASE MICROSCOPE
I. THE FORMATION OF "BLISTERS" ON CELLS IN SUSPENSION (POTOCYTOSIS), WITH OBSERVATIONS ON THE NATURE OF THE CELLULAR MEMBRANE *

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In a study of various tissue cells by means of the phase microscope (PM), a curious formation of "blisters" has been observed on several types of cells in suspension. The circumstances under which this phenomenon has been observed will be given in the present paper, along with certain implications which the findings may have in relation to the nature of the membranes surrounding cells.

THE PHASE MICROSCOPE

The PM equipment, first described by Zernike,[1] makes use of the following principle: If two lightbeams A and B (Text-Fig. 1) of the same wave length (λ) pass through two very thin glass plates, Ga and Gb, of the same thickness and refractive index, their original wave length becomes shorter because the refractive index of glass is higher than that of air. If the two beams enter the glass plates in the same phase of oscillation, they will leave it, and re-enter the air, still in parallel oscillations (A_1 and B_1). A third glass plate (Gc) of the same thickness, but darker and, therefore, absorbing a considerable amount of light, changes the amplitude c of a third lightbeam C into c_1, whereas the amplitudes of A and B will not be changed theoretically by the plates Ga and Gb, because these plates absorb no light. The effect of lightbeam A_1 on the eye is the same as that of B_1, while lightbeam C_1 appears less bright. A fourth beam D, going through a slightly thicker glass plate (Gd) will leave it in another phase of oscillation than the lightbeams A_1, B_1, and C_1 as seen in level X; there is a so-called "phase-difference" (PD) present. The same thing happens if light passes through small particles, which have a different index of refraction or a different thickness than the surrounding medium, as for instance in the case of mitochondria in protoplasm.

Unfortunately, the eye cannot recognize phase-differences in the ordinary microscope, but only differences in wave length (colors) and in intensity. The PM converts phase-differences into differences in amplitude by means of a "phase plate"; thus, it enables the human eye to perceive phase-differences as black and white contrasts. (For the theoretic explanation of the effect of the "phase plate" see Zernike,[1] Ganz,[2] and Bennett, Jupnik, Osterberg, and Richards.[3]) Therefore, the image can be observed with the PM without staining.

In practice, a number of intracellular constituents that are invisible or indistinct when studied by ordinary, darkfield, or ultraviolet microscopy have become readily visible in detail when examined with the

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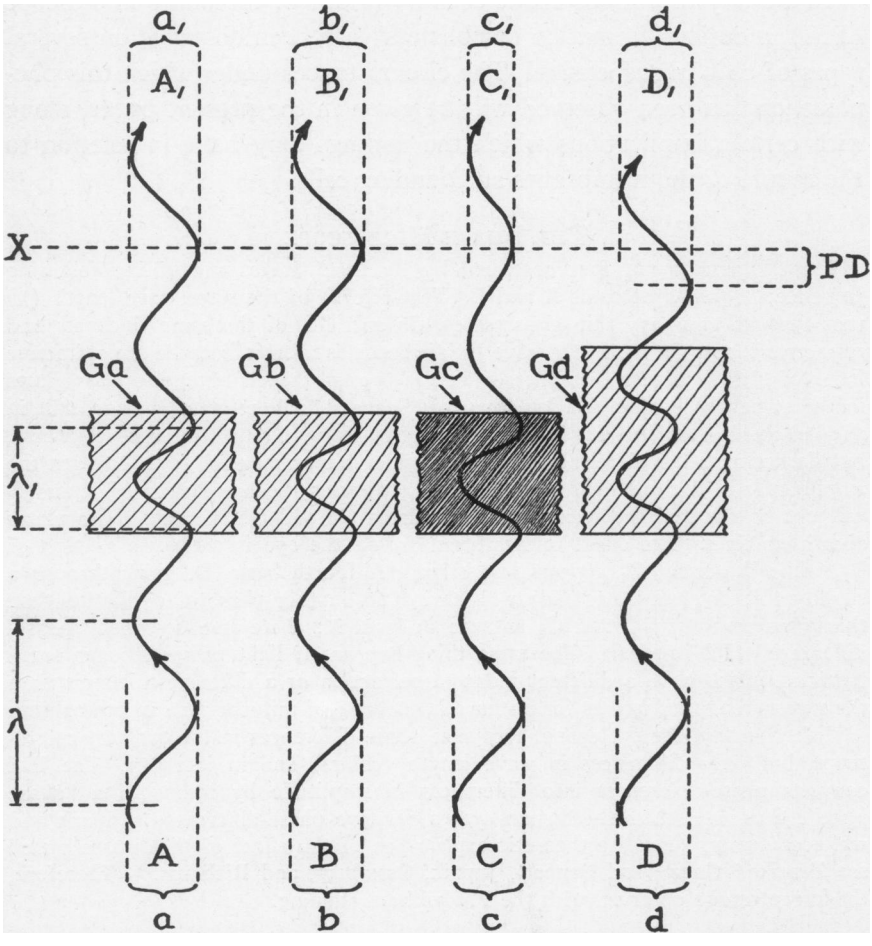
PM. To provide a comparative illustration of the results obtained with an ordinary and a phase microscope, photographs were made of the cells of an adenocarcinoma of the stomach as viewed in various preparations with the two kinds of optical systems (Figs. 1 to 4). Furthermore, by means of the PM the investigator can follow consecutive intracellular changes for hours and even days in tissue cultures and thus obtain, so to speak, a "longitudinal section" through the whole



Text-Figure 1. Schematic drawing illustrating the effect of different glass plates on light beams which are in the same phase of oscillation. Ga and Gb are made of colorless glass of the same thickness as Gc (dark glass). Gd is made of colorless glass, but it is slightly thicker than the other three plates. This difference in thickness causes a phase-difference (PD) of the beam D.

course of a process, whereas the microscopic section shows but a "transverse section" through the process at the moment when the cell

was killed by fixation. On the other hand, the microscopist should be mindful of the fact that effective phase microscopy depends upon small differences in refraction or thickness between objects. Finally, the fact deserves emphasis that the resolving power of the PM theoretically cannot be higher than that of an ordinary microscope; nevertheless, it is possible to see elements with the PM which are invisible, owing to shrinkage caused by fixation and dehydration, in stained preparations examined with the ordinary microscope.

Methods

The observations were made with a Zeiss instrument consisting of a centerable condenser with a ring-shaped diaphragm for each objective. The three objectives contained the phase plates. The photographs were taken with a 35 mm. "Alpa-Reflex" camera on Kodak Panatomic X film. As the cells floated freely in fluid, there was always a certain movement under the coverslip; hence photographs were rather difficult to take. Another difficulty was caused by the fact that the cells are three-dimensional. Thus, a certain level of a cell, actually in focus, may still appear indistinct because of the interference caused by elements in other levels.

Suspensions of normal cells of various types (kidney, liver, adrenal, stomach, and small intestine) procured from various animals (frog, rabbit, and mouse) were made by teasing, or washing, or scraping normal organs immediately after the animal was killed. Kidney cells of the frog proved especially suited to study with the PM, because they are not easily influenced by temperature, and they seem to survive the death of the animal for long periods (W. Lewis and McCoy[4]). In order to obtain suspensions of living tumor cells (Gardner's lymphosarcoma, C_3H sarcoma, and granulosa cell tumors of mice, V2 and Brown-Pearce carcinomas of rabbits), pieces of the neoplastic tissues were removed with aseptic precautions immediately after the animal was killed, thoroughly freed from as much of the adjacent normal tissue as possible, dissected in small pieces, and passed through a 40 mesh Monel-metal sieve.

Physiologic saline (0.9 per cent) and buffered Ringer's solution containing 150 mg. per cent of glucose (BGR) were used as suspension media. Liver cells of the frog required 0.5 per cent of NaCl (Anitschkow[5]), whereas a concentration of 1.25 per cent of NaCl is considered by von Möllendorff[6] to be physiologic for kidney cells. In order to watch the effects of various chemical agents, the cells were observed while the original suspension medium was replaced in the following

way: A drop of the chemical solution to be tested was placed on one edge of the coverslip and was drawn under the coverslip by means of filter paper placed at the opposite edge of the coverslip, which drew off the excess fluid. Of course, cells also were removed by this procedure, but there still remained a considerable number of cells in the microscopic field adhering either to the coverslip or the slide. During this replacement the cells were constantly observed with the high-power oil-immersion objective; the addition of a small amount of neutral red to the test solution facilitates the determination of the exact moment when the chemical reaches the cells under observation.

Each finding here reported has been confirmed by repeated observations in which different types of cells were used.

Observations

Various distinctive constituents of living unstained cells can be identified readily by means of the PM (Figs. 5 and 7). In undamaged cells, there usually is seen a distinct single-contoured, thin, cellular membrane, no matter whether the cell is isolated (Figs. 5 and 7) or located in the center of a small piece of tissue (Fig. 6). By means of this purely optical method it is impossible to distinguish between the plasma membrane and the extraneous cellular membrane (Chambers [7]).

The Formation of Blisters by Cells of Various Types in Suspensions

Cells in suspensions very often exhibit large "blebs" or "blisters" on their edges (Fig. 8). This phenomenon occurs in almost every kind of cell after the elements have remained in BGR or in isotonic salt solutions of other types for several minutes. In very fresh suspensions of the various cells studied, no blisters could be observed. The only cells that did not form blisters in these experiments were squamous cells from the mouth and tongue of man and frog. In the Shope papilloma only the basal cells showed blister formation.

The first signs of blister formation appear as early as 3 or 4 minutes after the suspension is made: small "cavities" arise in the protoplasm. They are usually adjacent to the cellular membrane, and later, as they grow in size, the cellular membrane bulges out until a blister is formed (Figs. 9 and 10). Then, the blister enlarges chiefly in width, thus detaching the adjacent membrane (Figs. 11 and 12), and finally the whole cytoplasm is surrounded by the contents of the blister, which separate the cytoplasm from the membrane (Fig. 13). Besides this type of blister formation, which may be called "diffuse," there occurs a "local" type (Fig. 14). In this, the base of the blister extends for

only a limited distance around the cell, and, after a time, the blister becomes more rounded. Since these two types can be observed in the same suspension, it does not seem that there is a fundamental difference between them.

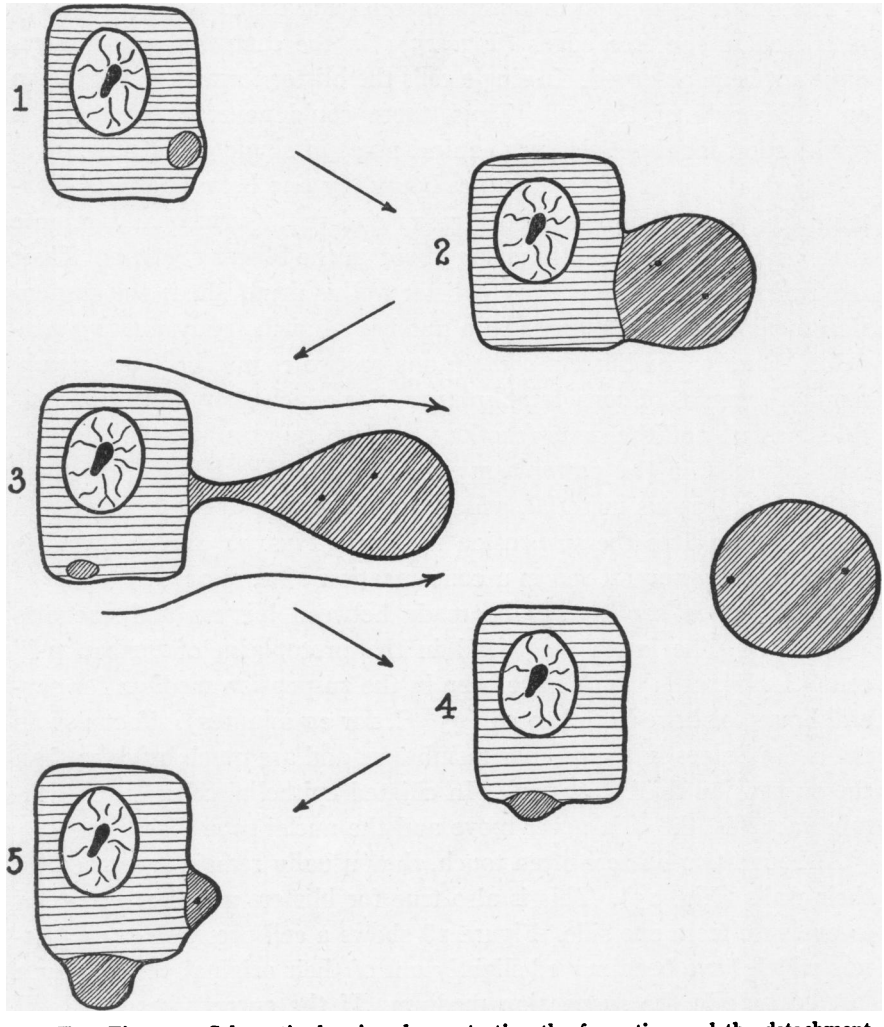
The blister formation in clumps of cells and tissue pieces is usually restricted to the free edges (Fig. 15); in the central region blisters have not been observed. In single cells the blister formation may begin on either side of the cell. Thus, there could never be observed a predilection for the inner face (apical pole) of single gland cells.

Figure 16 shows a very distinct boundary line between blister contents and protoplasm (see also Figs. 11 and 12). Occasionally, minute dark particles can be seen moving about in the blister contents. Their movement continues even if the blister wall is demolished; the suspension medium as a rule contains a number of such freely floating particles. Rarely, the blisters contain one or two round, brilliant, slowly moving granules of considerably larger size which show the same optical structure and the same reactions to chemical agents as do the brilliant granules in the protoplasm (see below). The blisters are filled with a homogenous material, which usually appears slightly darker in the PM than does the suspension medium (Figs. 11 and 12), and is not stained by neutral red in a concentration of 1:10,000.

A distinction can readily be made between blisters and vacuoles (Fig. 17). The latter are found in the protoplasm of "exhausted" cells, that is, cells which have been in the suspension medium for several hours, or have been heated (45° C. for 20 minutes). Vacuoles do not cause bulges in the cellular membrane, and are much brighter than the suspension fluid (Fig. 17). In ciliated epithelial cells, which contain vacuoles, the cilia never move and the nuclei soon disintegrate.

Although two blisters often touch, they usually remain separated by their walls (Fig. 15). This is also true for blisters which are exposed to pressure from one side. Figure 18 shows a cell presenting two blisters which have been moved slightly out of their original position by a fast current in the suspension medium. If the current becomes still more vigorous, the blisters are detached suddenly, and float freely in the suspension fluid. Text-Figure 2 shows schematically the entire process of blister detachment. Just before detachment, the blister becomes drop-shaped and the stalk appears more and more elongated until finally it is torn off. As soon as the blister is detached, it becomes spherical (Text-Fig. 2 and Fig. 19) and behaves like a rubber ball, its surface being momentarily indented if it bounces against an obstacle or a cell in the moving medium, immediately afterwards becoming

spherical again. Strong pressure on the coverslip divides free blisters into multiple small blisters. In detached blisters the black particles enlarge slowly, whereas the occasional brilliant granules do not change their size.



Text-Figure 2. Schematic drawing demonstrating the formation and the detachment of a blister. (1) Blister formation starts in the form of a submembraneous round space in the protoplasm. (2) A big blister with two granules has developed. (3) A strong current of the suspension medium (arrows) stretches the blister which is now drop-shaped. (4) The blister is detached; no defect of the membrane is visible. (5) Formation of a new blister starts again.

It is interesting to note that the cellular part of the blister stalk becomes flattened out immediately after the blister is detached, and a new blister is formed at the same place where the original one was

located (Fig. 20). Under these circumstances, neither the formation of a hole in the cellular membrane nor the outflow of the protoplasm has been observed. The site of the origin of the detached blister becomes completely invisible. Occasionally, new blisters are formed in the base of an already existing blister. These newly formed daughter-blisters grow into the old blister (Fig. 21), and some of them even may develop in the wall of the mother-blister, especially under the influence of certain chemicals (see below).

Experimental Alterations in the Process of Blister Formation

In order to determine the influence of the suspension medium on blister formation, suspensions of Brown-Pearce and V2 carcinoma cells were studied in normal rabbit serum and rabbit plasma as well as in BGR and isotonic salt solution. The same was done with frog cells of various kinds in frog serum, and the artificial mediums mentioned above. Blisters developed in all of these tests, although somewhat faster in the cells suspended in physiologic saline solution than in those in serum and plasma. There was no other difference in the process of blister formation in the various suspensions.

The cellular membrane disappears in cells suspended in 0.1 to 0.5 M ammonia. If, at the commencement of the experiment, blisters are present (Fig. 22), they are filled up by the swollen protoplasm and the nucleus as soon as 0.05 M ammonia reaches the cells, but the membrane remains visible (Fig. 23). Later, new blister formation may start in such a cell. The cellular membrane is not dissolved by 0.05 M ammonia, but swells, and sometimes daughter-blisters are formed within the membrane itself (Fig. 24). The formation of blisters is relatively independent of the pH of the medium (pH 2.3 to 10.0), but in a medium of pH 10.2 and higher, the membrane disappears and the contents of the blister flow out.

The cellular membrane very often becomes indistinctly outlined in dilute acetic acid, and the slightest pressure on the coverslip is sufficient to destroy the membrane. This change is irreversible. The ciliated epithelial cells show a particular form of reaction to acetic acid (Fig. 25): the cilia, instead of being straight and parallel, become irregular in form and arrangement. The basal bodies appear very dark, and occasionally the entire row of the basal bodies is separated from the rest of the protoplasm by a bright halo (Fig. 25). Therefore, the bases of the cilia behave like an independent section of the cellular membrane which does not shrink as much as does the rest of the membrane.

The formation of new blisters and their further growth is greatly

accelerated by distilled water (Figs. 19, 26, and 27). At the same moment that the distilled water reaches the cells under the coverslip, the nucleus and, to a lesser degree, the protoplasm swell so much that the whole blister is filled up in a few seconds. An instant later, the formation of new blisters starts. The black granules in such blisters swell considerably, particularly in detached blisters (Figs. 19 and 26). By this method it is possible to produce blisters even in cells in mitosis (Fig. 28), although they rarely develop in such cells in physiologic saline solution or in BGR.

Hypertonic salt solution, 0.9 per cent saline of pH 4.0, 70 and 95 per cent alcohol, and acetone, cause a very rapid shrinkage of the blisters, which goes on until the blisters have disappeared and the blister wall lies close to the protoplasm. In the course of this shrinkage, the blister wall becomes slightly wrinkled. In hypertonic salt solution, new blisters appear several seconds later; they grow very rapidly for several seconds and are morphologically indistinguishable from blisters in fresh cells. In the other media mentioned above, no further blister formation occurs, and even the replacement of the chemicals by physiologic saline solution does not produce a detachment of the cellular membrane.

The temperature of the suspension does not play a rôle in the process of blister formation: the amount and the size of the blisters are the same whether suspensions of the same mammalian cells are kept in the ice box, or at 37° or 41° C.

In cells which die spontaneously in the suspension, and in cells killed by heat or by chemicals, blister formation never occurs. Blisters, which are already present before the cells die, do not disappear, but they show no further growth. They remain visible until the cells disintegrate.

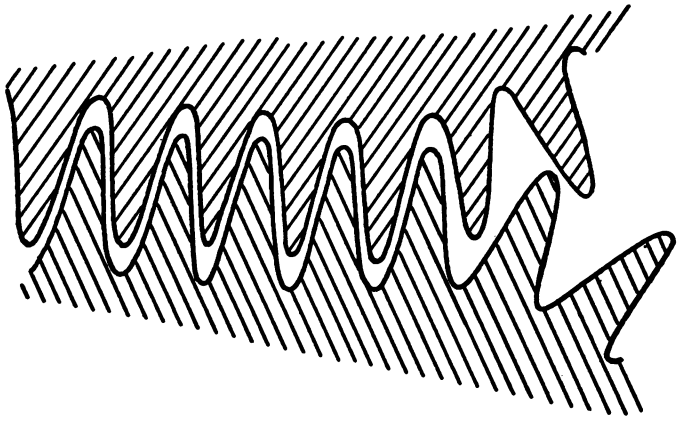
The Extrusion of Blisters from Renal Epithelium

Suspensions of kidney cells occasionally may contain some intact tubules held together by the basement membrane, in which case blisters develop only on the interior surface of the epithelium. The blisters grow very rapidly, and old blisters are very soon detached by the formation of new ones. Thus, free blisters are continuously expelled through the open ends of the tubules (Fig. 29). The same process was observed in unstained frozen sections of living kidney tissue, prepared from a human kidney removed surgically. The unfixed tissue was immediately put into physiologic saline solution and sectioned by means of a freezing microtome with a special knife-cooling device. The

sections were mounted in physiologic saline solution, and the edges of the coverslip sealed with vaseline. Slight, but distinct blister formation was visible in some of the tubules after a few seconds (Fig. 30).

The Cellular Membrane of Squamous Epithelium

Squamous cells were found to have a particular kind of cellular membrane. Suspensions of squamous cells were obtained by scraping the surface of the investigator's oral epithelium with the edge of a coverslip (Bosshard [8] and von Albertini [9]). The membrane of these



Text-Figure 3. Schematic drawing of the interdigitating wrinkles of the surface of two squamous epithelial cells. On the right is shown the artificial detachment of the two cells.

cells was rather thick and very stiff, thus maintaining the irregular shape of the cells. The membrane was dissolved by 0.5 M ammonia after 1 or 2 hours, whereas the other chemicals mentioned above did not cause any alteration of the membrane. The surface of these cells is delicately wrinkled (von Albertini) and its pattern resembles a fingerprint (Fig. 32), whereas the contour is serrated (Fig. 31). The height of the wrinkles is about 0.5 μ, their width 0.1 or 0.2 μ. The distance between two wrinkles is approximately 0.3 μ. As far as I could see, these wrinkles are folds of the superficial layer of the cellular membrane, interdigitating with those of the adjacent cells (Text-Fig. 3).

Discussion

Meltzer,[10] on theoretical grounds, introduced the expression "potocytosis" for the drinking or sipping of submicroscopic quantities of

water by cells. Later, the term "pinocytosis" was used to describe the intake of whole drops of fluid by "ruffle cellular pseudopodia" in tissue cultures (W. Lewis [11]). The word "potocytosis" seems preferable for the process of blister formation described in the present paper since an intake of entire drops of fluid by pseudopodia was not observed.

The observations described in this paper demonstrate that potocytosis is a very common process in cells in suspensions. It occurs in epithelial as well as in mesenchymal cells and in normal cells as well as in tumor cells. Squamous cells were the only type which did not show potocytosis. Even distilled water, and 0.05 M ammonia, which increase blister formation greatly in other cells, did not bring about potocytosis in squamous cells.

A process very similar to blister formation was described by Hogue [12] in cells of tissue cultures that were exposed to hypertonic salt solution. However, sometimes blisters developed even in normal Locke-Lewis solution. The detachment of blisters also was observed in Hogue's experiments. Margaret Lewis [13] described the formation of "blebs" along the edges of tissue cultures exposed to alkali. Similar "blebs" or sacs containing moving granules were observed by the same author in dying cells. These findings indicate that blister formation is not restricted to cells in suspensions.

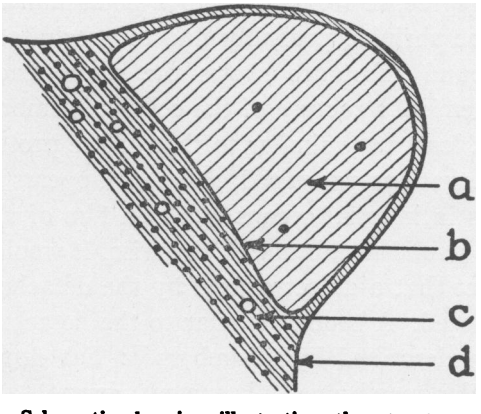
Since blisters fail to develop in cells which are completely surrounded by other cells in tissues, potocytosis must be considered a special type of fluid intake by living cells, which occurs particularly under unnatural conditions, *i.e.*, in suspensions and on the edges of tissue cultures. Therefore, it would appear that one of the fundamental conditions for potocytosis is the presence of a free cell surface in direct contact with an excess of fluid.

Furthermore, the formation and the enlargement of blisters are dependent on a second factor—the viability of the cells—for the enlargement and the new formation of blisters stop immediately when the cells die. This fact indicates that blister formation is an active function of the living cell and even a very low osmotic pressure of the medium is not sufficient to produce blisters in dead cells.

There is only a quantitative difference in the process of blister formation in cells in physiologic saline and those in hypertonic saline solution, distilled water, or homologous and heterologous serum. Hence the chemical constitution of the suspension medium seems not to be of fundamental importance for the process of potocytosis. The acceleration of blister formation in distilled water probably is a consequence of the greater osmotic pressure of the protoplasm as compared with

that of the medium. Under inverse circumstances, the cells being in molar NaCl, the blisters disappear due to the negative osmotic pressure. The subsequent rapid new formation and enlargement of blisters, which at first seem to contradict this explanation, are probably due to an increase in the permeability of the cellular membrane caused by a change in it. The process of blister formation was independent of the temperature of the suspension between 8° and 45° C.

The contents of the blisters seem to be part of the cellular protoplasm in sol-form, whereas the rest of the protoplasm consists of the different granules embedded in the ground substance, which here is a colloid in polyphasic form. Therefore, the granules originally located in the jelled cortex of the protoplasm (Chambers [7]), which is dissolved during the course of potocytosis (gel→sol), are the only ones moving freely in the blister contents later on. This fact proves that the distinct line of separation between the blister contents and the rest of the protoplasm (Fig. 16) is not a proper solid membrane, but a newly formed interface membrane. Therefore, the blister must be considered to be part of the protoplasm, and the blister contents to be outlined by an interface membrane (Text-Fig. 4). The free part of the blister wall behaves optically, as well as in its reaction to chemicals, like the



Text-Figure 4. Schematic drawing illustrating the structure of a cellular blister. (a) Blister contents with a few granules. (b) Internal interface membrane. (c) Protoplasm of the cell. (d) External interface membrane of the blister (plasma membrane).

cellular membrane in blister-free cells. It must be assumed that in regard to their physical and chemical structure these two membranes are identical.

The ability of living cells to form blisters is very interesting in regard to the problem of glandular secretion, for, although the conditions in the experiments reported above are unnatural, it is conceivable that

the secretion of at least some glands takes place in a manner very similar to potocytosis. A process resembling the formation and the detachment of blisters in suspensions has been observed by Jackson [14] in cells of renal tubules of rats which had been fed a very high-protein diet. This author considered blister formation *in vivo* to be a sign of cell injury. The process is not restricted to kidney cells, since Jackson also saw it in uterine glands during pregnancy. Furthermore, the formation of blisters in renal tubules as observed in frozen sections of living tissue is similar to the process of "granuloid formation" in renal tubules (Kosugi [15]). Bell's assumption [16] that the "granuloid" is an artifact of extracellular origin is disproved by the above-mentioned observations (see Fig. 30). Although we know the blisters to be a product of living cells, it is not possible at the present time to decide whether potocytosis in kidney cells is restricted to cells surviving the death of the individual or whether it occurs, either under physiologic or under pathologic conditions, while the individual as a whole is alive.

The phenomenon of potocytosis and the reaction of the cellular membrane to various chemicals aid in forming conclusions concerning the nature of the cellular membrane. NaOH (pH 10.2), and 0.1 to 0.5 M ammonia dissolve the cellular membrane, whereas in alcohol, acetone, and, to a lesser degree, in formalin, and in potassium bichromate, the cellular membrane shrinks, probably due to precipitation. Since the protoplasmic ground substance shows the same reactions to these chemicals, the chemical structure of the cellular membrane is likely to be identical or at least very similar to that of the protoplasm.

From the observations presented above, it appears that the membrane of the cells in internal organs is a thin film of a slightly sticky fluid rather than a solid membrane. Otherwise, it would be impossible to explain the fact that a defect caused by the detachment of blisters is immediately closed. A process similar to the detachment of blisters already has been described by Chambers [17]: Fat droplets may pass through the cellular membrane (plasma membrane) after intraprotoplasmic injection without causing a visible defect in the membrane. Danielli,[18] using an oil-water interface model, was able to demonstrate the same phenomenon, thus assuming the cellular membrane to be merely an interface membrane. The cellular membrane does not behave like a semipermeable membrane either; the black and the brilliant granules are influenced and changed by hypertonic as well as by hypotonic salt solutions (Fig. 19), thus proving that the membrane is permeable to salts.

The phenomenon of "pinocytosis" (W. Lewis [11]) is another proof that the cellular membrane cannot be solid. Therefore, the plasma membrane, which represents the only cover of the majority of the cells of the inner organs and of many tumors, has to be considered a simple interface membrane between the cytoplasm and the surrounding medium. The observation that blisters usually do not merge may be interpreted as a consequence of their surface tension. The same force prevents the blisters from rupturing.

The stiff membrane of the squamous cells seems to be identical with the "extraneous cellular integument," Chamber's "proper cellular membrane," [7] although I was not able to distinguish it optically from the hypothetic underlying plasma membrane. The observation that these cells fail to develop blisters suggests that the lack of an extraneous cellular integument is a third conditional factor for the formation of blisters.

Summary and Conclusions

Potocytosis, the process whereby visible blisters form on tissue cells suspended in liquid media, has been described in detail. Squamous cells alone amongst those studied did not exhibit blister formation, whereas this was almost invariably seen in normal and neoplastic cells of other types when one or more of their surfaces had remained in contact with an excess of fluid for a few minutes. The process of blister formation was accelerated in distilled water and in hypertonic solutions. The blister contents seemed to consist of highly diluted protoplasmic ground substance which was separated from the rest of the protoplasm by an interface membrane. When blisters were detached, the cellular membrane remained apparently intact. It is conceivable that some glandular cells pour out their secretions by the detachment of blisters, and that the "granuloids" seen in renal tubules by Kosugi [15] and others may be detached blisters.

The findings as a whole seem to support the theory advanced by others that many of the cells of the internal organs and those of some tumors are outlined by an interface membrane that lies between the protoplasm and the surrounding medium. Furthermore, the permeability of the cellular membrane to hypotonic and hypertonic saline solutions, as indicated by visible changes in the cytoplasm of cells suspended therein, seems to be much greater than is commonly supposed. The integument of squamous cells, by contrast, seems to be a genuine stiff membrane which is folded into minute wrinkles that interdigitate with those of adjacent cells.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 100

FIGS. 1 to 4 show various preparations of human tumor material (adenocarcinoma of the stomach, biopsy) as seen with the ordinary and the phase microscope (PM). × 700.

FIG. 1. Dry smear stained with Giemsa stain; ordinary microscope. The nuclear and protoplasmic elements are very indistinct and the cells are markedly shrunken.

FIG. 2. The edge of an unstained frozen section of fixed material; PM. The nuclear constituents are readily visible, whereas the protoplasm, due to the formalin effect, is granular.

FIG. 3. Paraffin section of Zenker-fixed material; hematoxylin and eosin. The cells have shrunk and the nuclei are irregularly outlined. The nuclear elements are less distinct than in Figure 2; the protoplasmic structure is about the same. (Ordinary microscope.)

FIG. 4. Unstained cells in suspension; PM. The cells are not shrunken, the elements of the nuclei and the protoplasm being readily visible. *a* demonstrates a giant nucleus, *b* shows a multinucleated cell, and *c* illustrates a mitotic cell (metaphase).

FIG. 5. Fresh suspension of frog kidney cells. The elements are distinctly outlined by the cellular membranes. The protoplasm is filled with small, dark granules and the nuclei are "clear," containing a few dark dots. PM. × 700.

FIG. 6. Piece of a renal tubule of a frog. The cellular membranes are easily seen, while numerous small, black granules are visible in the cytoplasm of all of the cells. In the peripheral cells they are round and enlarged, while in the central area they are more rod-like. PM. × 700.

FIG. 7. Cylindrical cell of the stomach, surgical specimen. There are three types of protoplasmic granules visible in the homogenous ground substance: (1) five large, white, spherical droplets on the left of the nucleus; (2) a group of very small, black granules in the apical pole (on the left) and near the base of the cell (on the right); and (3) many dull-gray granules in the basal part. PM. × 1400.

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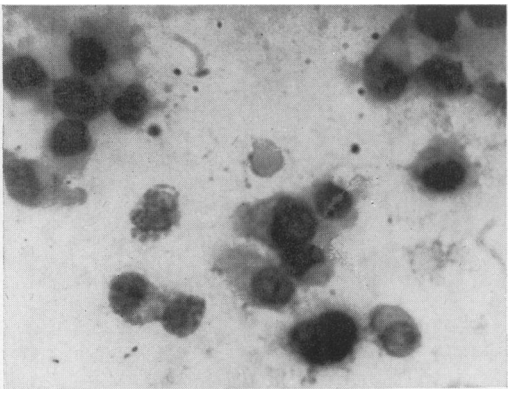

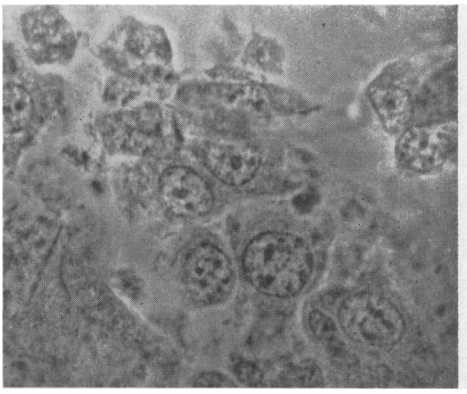

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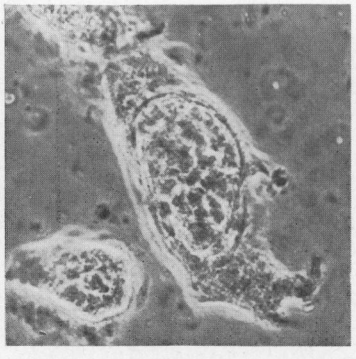

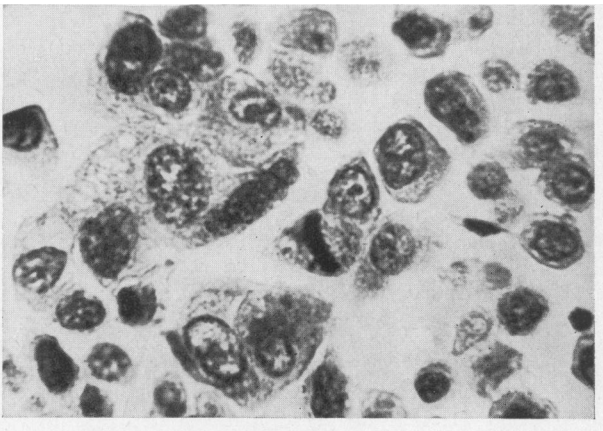

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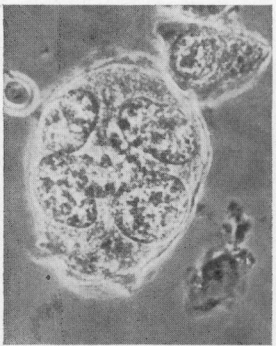

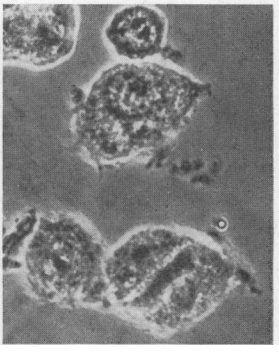

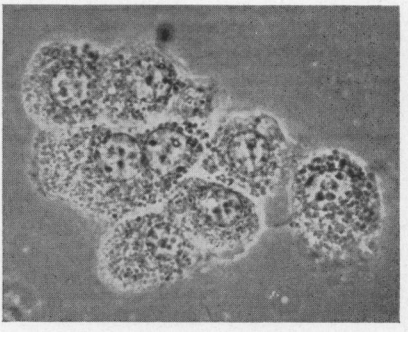

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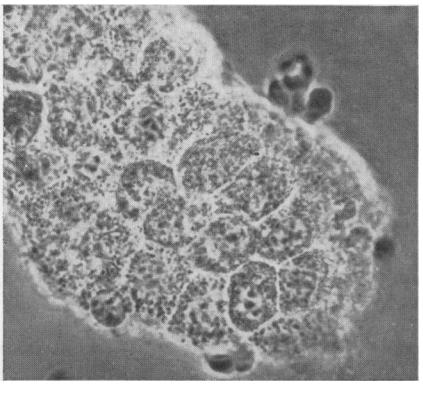

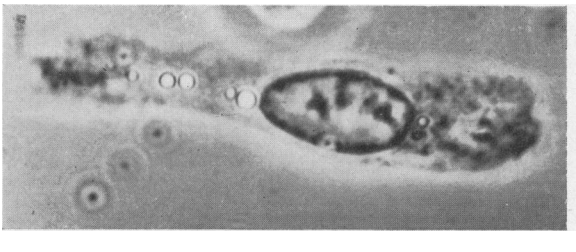

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Phase Microscopy, Potocytosis

PLATE 101

FIG. 8. Brown-Pearce carcinoma cells, after having been in the suspension under the coverslip for 30 minutes, showing extensive blister formation. In the right middle cell there are several small blisters in the contents of a large blister. Above this cell there is a free blister. The process of detachment of a blister can be seen in its terminal phase below the cell just mentioned. PM. × 700.

FIG. 9. Intraprotoplasmic origin of a blister (on the right) in a kidney cell of the frog, 4 minutes after the animal was killed and the suspension was made. PM. × 1400.

FIG. 10. Brown-Pearce carcinoma cell containing one intraplasmic and one bulging blister. PM. × 1400.

FIG. 11. V2 carcinoma cell showing diffuse blister formation. The protoplasm is compressed by the blister contents; the latter appear slightly darker than the suspension medium. The blister is surrounded by a bright diffraction ring. PM. × 1400.

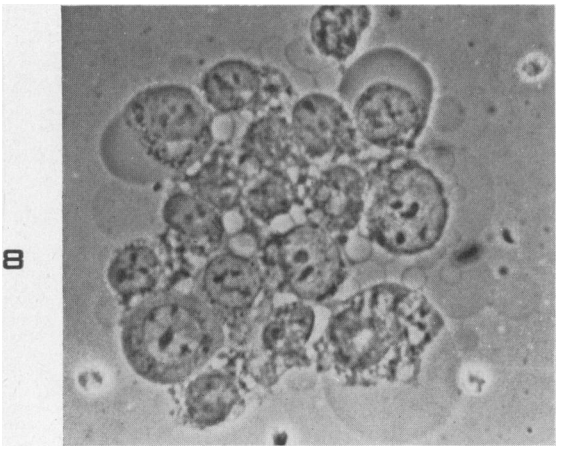
FIG. 12. The same cell as shown in Figure 11, 10 minutes later. The blister is markedly enlarged, and a second bright ring between the protoplasm and the blister contents has developed. PM. × 1400.

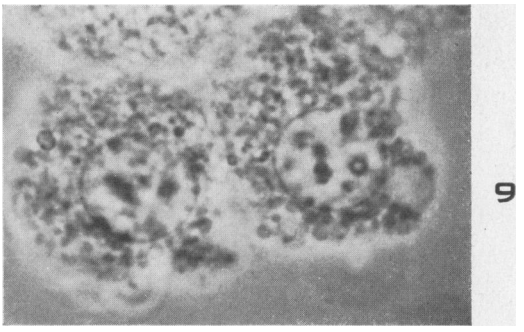
FIG. 13. Granulosa cell tumor showing marked diffuse blister formation after the suspension was in the ice box for 24 hours. The cell in the center, besides being "ballooned" diffusely, contains a spherical vacuole, the contents of which are much brighter than that of the blisters. PM. × 1400.

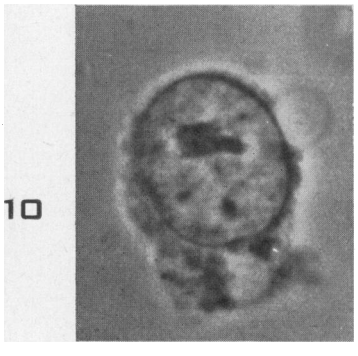
FIG. 14. Rabbit sarcoma cell exhibiting a single "local" blister. Of note is the distinct line between the blister contents and the rest of the protoplasm. PM. × 1400.

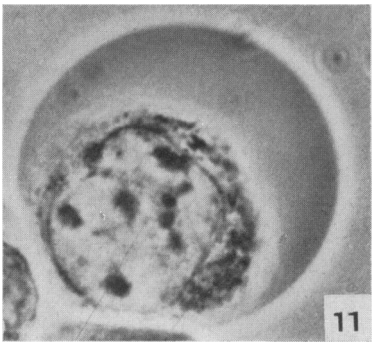
FIG. 15. Blister formation on the edge of a cortical piece of a frog kidney, 2 hours after the suspension was made. Some of the blisters are detached and stick on the surface of other blisters, but they do not merge. Several small granules are visible in some of the blisters. PM. × 850.

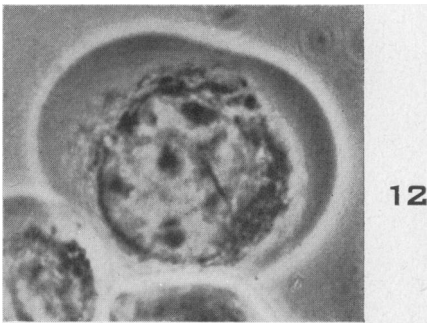
FIG. 16. Large blister in a kidney cell (frog) containing some relatively large, black granules (out of focus). The blister contents are separated from the compact protoplasm by a distinct line. PM. × 1400.

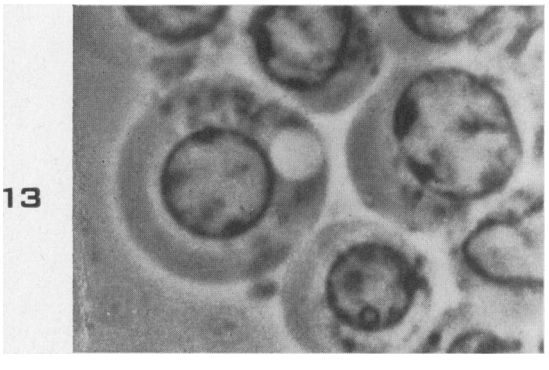


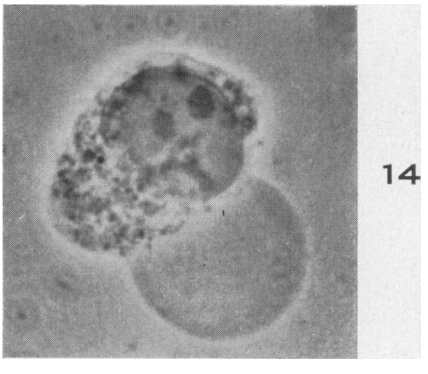


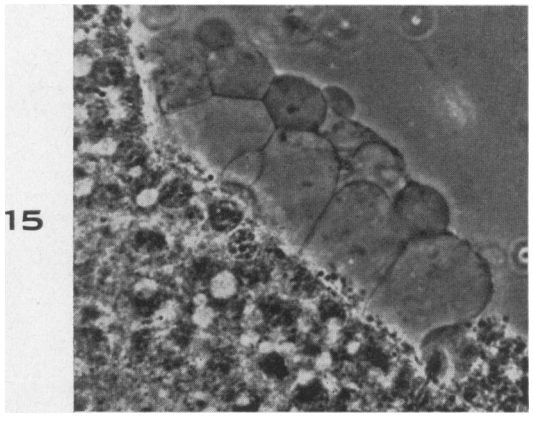


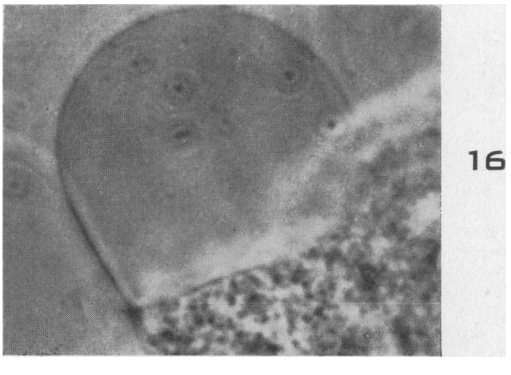












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Phase Microscopy, Potocytosis

PLATE 102

FIG. 17. Degenerated Brown-Pearce carcinoma cell after having been in the suspension at 37° C. for 5 hours. There are numerous small, bright vacuoles in the protoplasm. PM. × 1400.

FIG. 18. Brown-Pearce carcinoma cell. An artificial current of the suspension medium under the coverslip from the left to the right deflects two blisters, but they do not merge. PM. × 1400.

FIG. 19. Artificial blister of a ciliated frog cell in distilled water. On the left is a detached blister, containing some enlarged dark granules. PM. × 1400.

FIG. 20. Kidney cells of the frog. Formation of a new blister may be seen in the cell on the left after a blister has been detached previously. The new blister again contains some black granules. PM. × 1400.

FIG. 21. Brown-Pearce carcinoma cell showing numerous blisters, two of which have developed within the contents of a large blister. PM. × 1400.

FIG. 22. Ciliated cells of the frog pharynx. The chromatin network is out of focus; large spontaneous blisters have developed. PM. × 1400.

FIG. 23. The same cell as shown in Figure 22, 3 minutes after ammonia has been added to the suspension. The cilia have begun to disintegrate, the small, black, intraprotoplasmic granules (see Fig. 22) are very indistinct. The nucleus is enlarged and its membrane has almost disappeared. PM. × 1400.

FIG. 24. Frog kidney cell in 0.05 M ammonia, showing a large blister. The blister was already present before the ammonia was added to the suspension, but the ammonia caused a marked enlargement of the nucleus, which now fills the whole blister. A small new blister has been formed within the blister wall. PM. × 1400.

FIG. 25. The effect of 5 per cent acetic acid on a ciliated cell of the frog pharynx. The cilia are curled and their basal bodies are detached as a whole from the rest of the protoplasm. The nuclear membrane is double contoured and brilliant. PM. × 1400.

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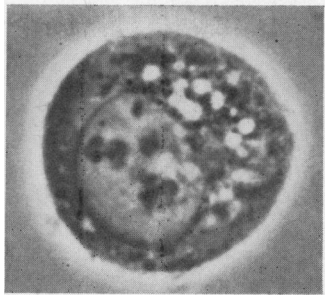

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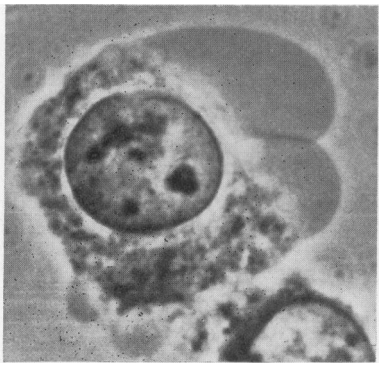

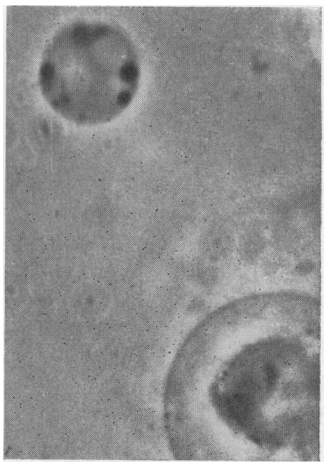

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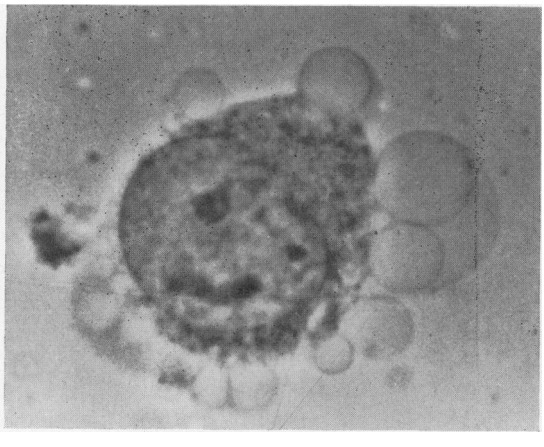

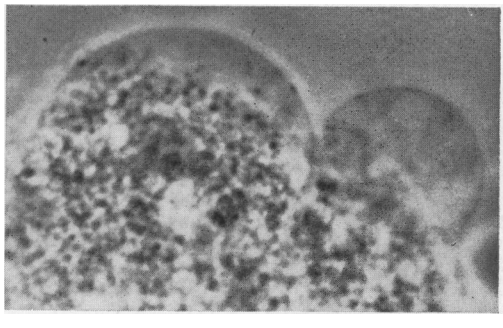

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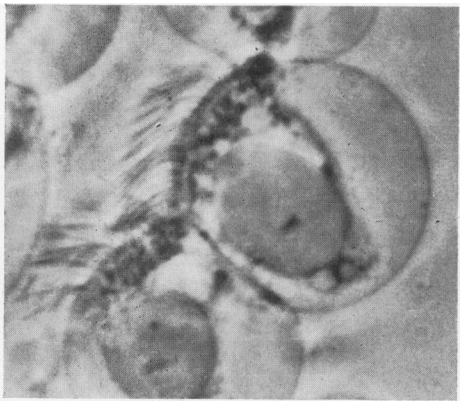

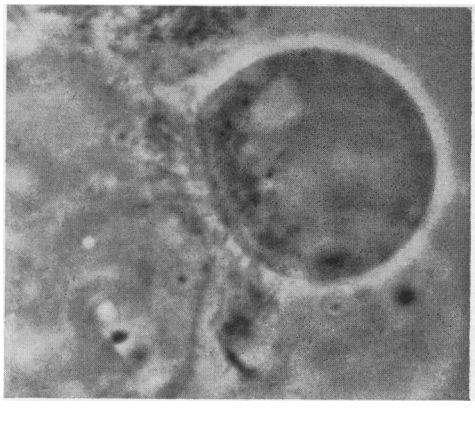

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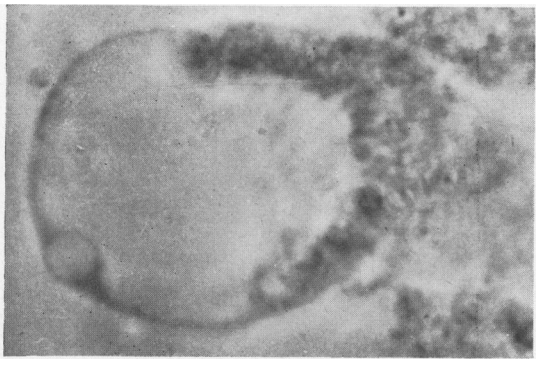

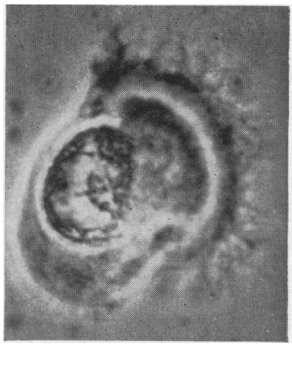

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Phase Microscopy, Potocytosis

PLATE 103

FIG. 26. Brown-Pearce carcinoma cells with artificial blisters, caused by distilled water. The entire cellular membrane, stretched out by a high intracellular tension, seems to form the blister wall. The nucleus on the right is hazy and homogenous, its nucleolus as well as the nucleus of the cell on the left are out of focus. The protoplasmic granules are enlarged. PM. × 1400.

FIG. 27. Artificial old blister of a C3H sarcoma cell, which has been in distilled water for one hour. There are some small vacuoles in the compressed protoplasm. PM. × 1400.

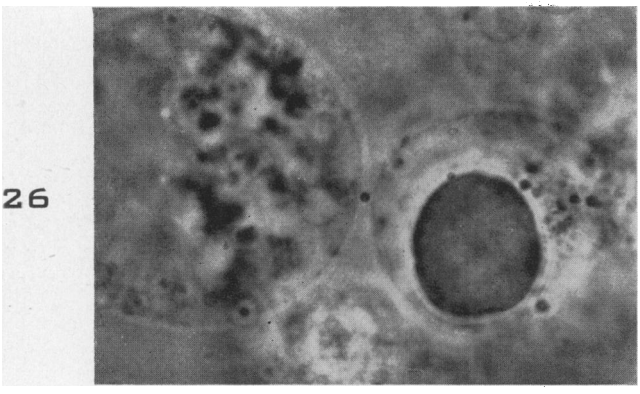
FIG. 28. Spontaneous blister formation in C3H sarcoma cells. The cell on the right is in mitosis and its blister shows stalk formation. A bright halo surrounds the chromosomes, and small, black particles are present in the protoplasm of this cell. PM. × 1400.

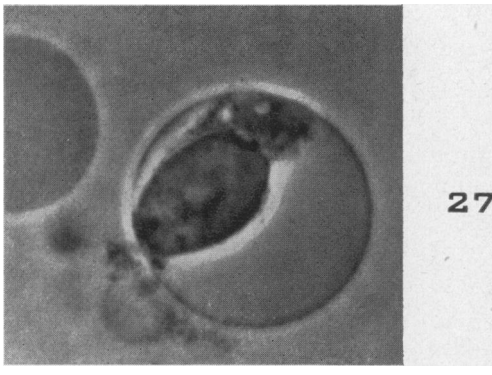
FIG. 29. An intact tubule of a mouse kidney has poured numerous blisters out of its open end. PM. × 700.

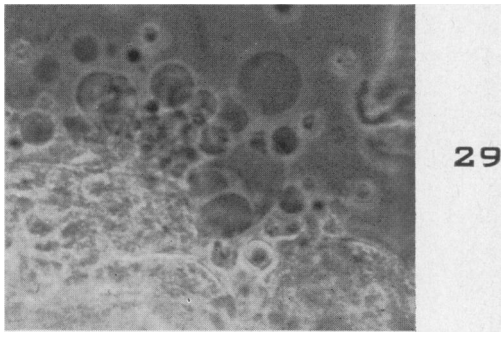
FIG. 30. Unstained and unfixed frozen section of the cortex of a human kidney in physiologic saline solution. The section was made immediately after the surgical removal of the organ: the cells are still living and show blister formation into the lumen of a tubule. PM. × 400.

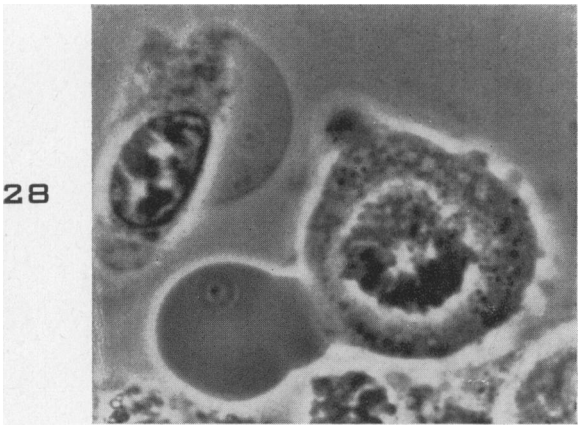
FIG. 31. Cells of the same cell type as shown in Figure 32, in profile. The wrinkles are rather high and very sharp; the protoplasm contains many irregular, dark elements. PM. × 1400.

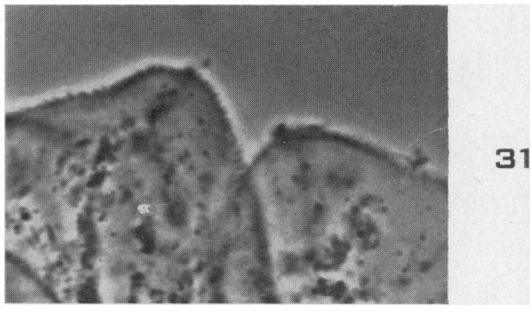
FIG. 32. Upper surface of a squamous cell of the human mouth. The delicate wrinkles are clearly visible, as are the sharp edges of the cell. Some bacilli stick on the cellular surface. PM. × 1400.

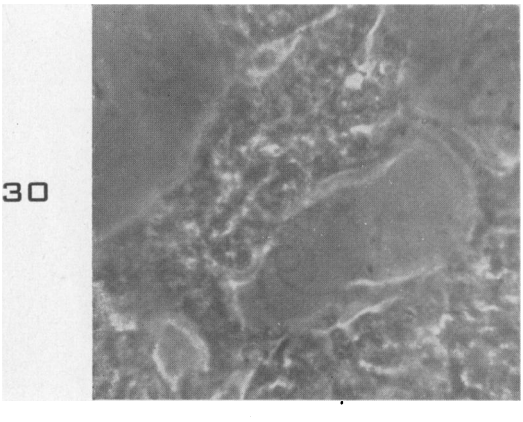


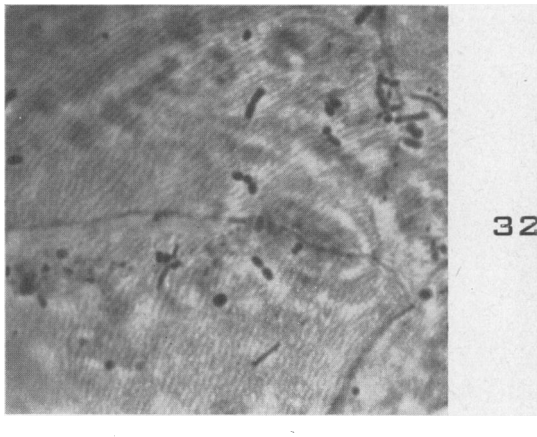












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