ing. As the material drops from the bearing into the receiver (9), it is transported by a current of inert gas through a return tube (7) to the reservoir (4) from which it may again flow between the shaft and bearing. This continuous circulation and regrinding is maintained until an examination of the material in the reservoir by the hanging drop or Gram stain methods shows that sufficient disintegration has occurred.

A number of details are essential to the success of the operation. A smooth bearing and shaft running without vibration at high speed have very little effect on the yeast cells. The surfaces of the bearing and shaft must be roughened by rubbing them with moist carborundum powder (100 mesh) before assembling the apparatus. About 0.1 g of the abrasive is added to 20 cc of suspension to insure keeping these surfaces rough. Vibration is produced by a vibrating joint. The occasional addition of a drop of octyl alcohol to prevent foaming is sometimes very effective in securing a relatively high concentration of the desired factors. Pyrex glass is used throughout to avoid the marked change in acidity which is caused by soft glass and to increase the life of the apparatus. It is desirable, although not essential, to permit the bearing to project part way into the liquid in the reservoir. An opening blown in this tube just above the stopper prevents any dead space. A current of nitrogen, air or other suitable gas is passed through a bubble counter and humidifier containing distilled water. A convenient rate is 3 to 5 bubbles per second; but when the shaft and bearing have become worn, a more rapid rate may be desirable to prevent the formation of any considerable column of liquid in the return tube. If gas bubbles up between the shaft and bearing, either the clearance between the two is too great or the current of gas has not been rapid enough to prevent accumulation of fluid in the receiver and return tube. The motor is run at 3,000 to 4,000 r.p.m.; the speed depends somewhat on the action of the vibrating joint, which should not become violent enough to damage the bearing.

The average length of run has been five hours. Examination showed that 50 to 75 per cent. of the cells had disappeared within 90 minutes and 90 per cent. in 300 minutes. The disintegration is most efficient with the more concentrated suspensions, since the rate at which the cells are ruptured depends apparently on the probability of a cell being pinched between the rotating, vibrating rod and the rough bearing surface. After centrifugation and filtration through a Berkefeld filter, the turbid sterile filtrate is used without undue delay. The apparatus may be used for disintegration of other biological materials and for securing intimate contact and mixing in chemical reactions involving combination of a gas with a suspended solid where continuous exposure of new surface is desired.

Explanation of the Diagram

The dimensions given are those of an apparatus which gave consistently satisfactory results, but there is no apparent reason why many of them could not be altered: (1) Motor—a "Sew motor" with its rheostat was used; (2) vibrating joint: a 2 inch length of glass tubing connected securely to the shaft of the motor with rubber tubing and to (3) with a two inch length of heavy rubber tubing; (3) disintegrator shaft, a straight rod or capillary tube 27 cm by 7.8 mm; (4) reservoir, a 12 cm by 3 cm glass tube attached to the bearing by a number 6 rubber stopper; (5) bearing, a straight tube 22 cm by 10 mm outside diameter, wall thickness, 1 mm; (6) return tube in three sections, 3 mm outside diameter; (7) gas outlet, identical with (6); (8) gas inlet; (9) receiver, constructed from a 6 x ¾ inch side arm test-tube either by sealing a 17 cm section of the return tube to the base as illustrated, or (10) by cutting off the base of the tube smoothly, flanging the edge, and using a number 4 stopper to connect the return tube.

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SPECIAL ARTICLES

THE DEVELOPMENT OF ORGANIZED VESSELS IN CULTURES OF BLOOD CELLS

In an experiment in which blood cells were placed for incubation in culture flasks containing a mixture of blood plasma and Tyrode solution, the usual technique for the cultivation of the leucocytes[1] was slightly modified in that coagulation was allowed to take place spontaneously and without the customary addition of embryonic tissue juice. Later, the cultures were found to contain numerous, highly organized, tubular processes that projected out from the original explant. A year ago, Hueper and Russell[2] reported

[1] A. Carrel and A. H. Ebeling, "Pure Cultures of Large Mononuclear Leucocytes," *Jour. Exp. Med.*, 36: 365, 1922.

[2] W. C. Hueper and M. A. Russell, "'Capillary-like Formations' in Tissue Cultures of Leucocytes," *Arch. f. exp. Zellforschung*, 12: 407, 1932.

the appearance of similar structures in a certain percentage of "leucocyte" cultures,[3] some of which had been prepared in the same manner as those referred to above. The phenomenon was but casually studied by them, however, and no attempt was made to determine the conditions under which it occurred. It seemed necessary, therefore, to study the matter anew and to ascertain the exact conditions under which the structures arise. It is the purpose of this communication to present the information thus far obtained.

The cells were taken from blood drawn from the carotid of adult chickens according to the procedures regularly employed in the preparation of plasma. After centrifugation, and the subsequent removal of the cell-free plasma yield, the thin superficial layer of plasma containing the buffy coat of leucocytes was coagulated by the addition of a drop or two of embryonic tissue juice. A few minutes later, this layer could then be removed as a disk and cut into small fragments for the preparation of the cultures. Each fragment consisted of a layer of white cells together with such red cells as remained adherent to them. In some cases, the red cells were separated, as completely as possible, from the fragments and discarded. In other cases, a considerable quantity of them was allowed to remain.

The fragments were placed in flasks to which plasma and Tyrode solution had previously been added. In a few instances, the plasma was replaced by serum, thereby eliminating all possibility of coagulation. In certain experiments in which plasma and Tyrode solution were used, a trace of embryonic tissue juice was added in order to induce rapid coagulation. In others, substances were introduced that would stimulate the cells to great activity without the immediate coagulation of the medium, as, *e.g.*, tryptic digest of fibrin. Some of the cultures comprising these various experiments were allowed to remain for a time at room temperature prior to incubation at 37° C. Others were placed in the incubator the moment they were prepared.

These experiments have disclosed the fact that the formation of the capillary-like structures is, in the beginning, a purely physical phenomenon dependent upon the response of the cells to gravity, the consistency of the medium, and the surface peculiarities of the individual cells. As soon as fragments containing both red cells and leucocytes have been placed in a plasma mixture that does not coagulate immediately, certain cells, in passing from a higher to a lower level, initiate a general outflow from the central mass into the surrounding medium. Red cells have smooth surfaces and easily shift their position. Leucocytes, on the other hand, being capable of independent locomotion, adhere to one another and to solid structures. They may, however, be swept along in a current of red cells.

The configuration assumed by the outflowing blood cells depends upon the consistency of the medium and the elevation of the fragment above the general level of that medium. If the medium is composed of serum, the cells flow out from all sides as when a vessel bursts that contains a viscous fluid. In the presence of plasma, however, the outflowing cells may take every conceivable form ranging from broad, fan-like disseminations, through short, stalky, bud-like projections to long, slender ones. It will not be possible at this time to describe more than one type of formation, namely, that which results in the subsequent development of a long, slender tube.

At one or more points on the margin, a few cells become dislodged and break away from the fragment. Their places are taken by those behind. If the proximal impact is great, these cells are also pushed out into the medium. A general streaming begins. Each cell that is forced out follows in the wake of those that have gone before. They may proceed in single file, or abreast of one another, but always over exactly the same route. This route assumes the nature of a tunnel-like passage through the plasma. As long as the cells in the lead are being pushed forward by those from behind, they will continue to advance through the medium until it coagulates. The moment coagulation occurs, however, the cells in the lead, being unable to make further progress, come to a stop. If coagulation occurs before the proximal outflow has ceased, the passage will remain open and unobstructed, despite the fact that the cells in transit may be widely separated If the pathway is only partially filled with cells, they may be made, at any time, to flow in either direction by manipulating the flask. After this happens, the force of the outflowing cells may still be so great that a large spherical expansion is formed at its distal end. Enlargements may also appear elsewhere along the tube. Very often it may be completely ruptured, and occasionally, short branches are found.

All this may occur within half an hour after the cultures have been prepared. As soon as the surrounding medium has become firmly coagulated, however, now further change occurs either in the length of the tube or in its diameter. The structures do not, as Hueper and Russell[2] have reported, continue to grow upon further incubation.

Up to this point, the development of the tubules depends solely upon the rapidity with which the plasma coagulates. A culture held at room tempera-

[3] Although not implied by the title of their report, these cultures contained every cell type present in the circulating blood.

ture develops tubules in greater number and of greater length than a sister culture incubated immediately. Their development is not suppressed by substances that enhance cell activity unless they induce, at the same time, the immediate coagulation of the medium. Embryonic tissue juice prevents their formation by producing immediate coagulation.

So far, the tubules have no definite walls. These begin to be formed very shortly after coagulation has taken place in the medium. The walls do not arise as a direct transformation of the fibrin clot. Instead, they are formed by the activity of living cells, or cell products, the so-called thrombocytes, that have been deposited along the course of the tubule. Where there are gaps between the cells confined in the tubule, these minute bodies may be seen to spin out fine hair-like filaments at the interphase between the lumen of the tubule and the surrounding coagulum. It is believed that the fibrin of the plasma clot serves as a supporting structure upon which the thrombocytes lay down their fiber-like strands. Out in the surrounding medium, these small corpuscles produce similar strands, but here at random. The majority of them tend to agglutinate. Very often, the agglutinated masses become joined together by numerous threads.

After a few days, the red cells within the capillary-like formations become progressively phagocytized by the macrophages. Eventually, these in turn escape into the surrounding medium, sometimes through definite breaks in the walls, but more often by way of their proximal ends. When the tube has become empty, as may occur after 4 or 5 days, any number of macrophages may remain spread out over its outer surface. In this position, they bear striking resemblance to the much discussed cells of Rouget. At times, they are so numerous that their undulating membranes seem to fuse with one another, giving the impression of an unbroken syncytium. If the cultures were fixed and stained at this moment, their nuclei might easily be mistaken for nuclei present in the wall. This apparently accounts for the statement of Hueper and Russell[2] that the wall itself becomes nucleated. This has not been confirmed. When, as may eventually happen, these Rouget-like cells wander away, they leave a fibrous wall that is quite devoid of any cellular structure whatsoever.

Occasionally, the wall is incompletely formed, the fibrillar strands being laid down over a single portion of the original pathway. At other times, a single cord of fibrous material may extend along one side of it, or even this may be absent. Invariably, however, its lumen remains open and filled with the clear fluid that has diffused in from the medium.

To summarize: Isolated blood cells, in a plasma substratum, are capable of constructing highly organized channels that are analogous to the blood capillaries of the organism.

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LAMINAR DESTRUCTION OF THE NERVE CELLS OF THE CEREBRAL CORTEX

ONE of the structural characteristics of the cerebral cortex is that it is composed of a number of layers of nerve cells. Generally a fundamental subdivision in six layers is accepted nowadays.

Very little is known as yet about the functions of these various layers. It is known that in the gigantopyramidal area of the cortex (field 4 of Brodmann) the giant pyramidal cells of the fifth layer give rise to most of the nerve fibers descending into the spinal cord as the fibers of the pyramidal tracts. It is assumed on the basis of histological evidence that the three or four outer layers are predominantly receptive in function and that the two inmost layers more particularly subserve efferent functions. Perhaps a few additional, although indeed less probable assumptions could be made, but that would be all. Our factual knowledge of the functions of these various layers is as yet very restricted. This is largely due to the stupendously complex structure of this tissue, but also to the fact that until now no method has been available which permitted an experimental attack upon the various layers of the cortex, and by that a direct approach to their respective functions.

In this preliminary paper I want to describe briefly a simple method which makes possible destruction, at will, of consecutive layers of the nerve cells of the cerebral cortex, *i.e.*, of either its first two superficial layers, or of the first four, or of all the six layers. This is possible by applying heat locally to the exposed cortex for a very short period. It was found that heating to 90°–100° C. for five seconds results in death of all the nerve cells in the heated area throughout the whole thickness of the cortex, and that, by heating it to about 70° C. for from one to two seconds, it is possible to kill the nerve cells of only the two superficial layers.

The apparatus used in these experiments is very simple. The copper tip of an ordinary electric soldering-iron is sawed off so that a rectangular surface of appropriate size (5 × 7 millimeters) is obtained. This surface can be heated to any temperature suitable for our purposes, by taking off the proper voltage from a potentiometer-rheostat (of f.i. 600 ohms, 1 Amp.) plugged into a power outlet. For special purposes copper tips of various size and shape are used, which can be screwed into the stem of the soldering-iron. A series of settings of the rheostat was established for a few suitable temperatures (60, 70, 80, 90, 100° C.) for each type of copper tip used. The temperatures of the tips were determined in the usual