INTERMITTENT TAKE-UP OF FLUID FROM THE CUTANEOUS TISSUE

By PHILIP D. McMASTER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

PLATES 1 AND 2

(Received for publication, September 30, 1940)

Lymph formation is greatly influenced by physical forces within the tissues. Earlier work from this laboratory has shown (1, 2) that the mechanical force of the pulse increases lymph formation and further (3–5), that the movement of interstitial fluid is enhanced by intermittent pressure transmitted from the pulse to connective tissue fibrils and other formed elements along or between which the fluid must pass to gain entrance into the lymphatics.

In the present work we have attempted to learn more about the formation of lymph by a further study of the effects of mechanical forces upon the movement of fluids within the tissues. We have measured by direct means changes occurring in the interstitial movement and in the absorption of fluids brought into immediate contact with the tissues of the skin of man and other living animals under a variety of physiological and pathological conditions. The changes in pressure to which these fluids were subjected in various tissue states and the concurrent effects upon interstitial fluid movement have also been determined directly.

Before these studies could be made certain difficulties had to be overcome. The interstitial movement and absorption of fluid brought into contact with the tissues of the skin cannot be measured if it is allowed to enter directly into either blood vessels or lymphatics. The pressure exerted by the tissues upon extravascular fluid cannot be determined if amounts of fluid large enough to be easily measurable by ordinary means are forced into a tissue, for there will result an artificial interstitial pressure incidental to the distortion of the formed elements. To avoid these difficulties we have devised methods whereby measured microscopic amounts of fluid can be brought into contact with the cutaneous tissue at atmospheric pressure or under positive pressure, in such a manner that fluid does not directly enter either the blood capillaries or the lymphatics. So minute are the amounts of fluid employed that its movement approximates the natural, as will be seen below, and artificial intercellular pressure is either avoided or created at

will for study. These methods will be described in the present paper together with some of the changes which occur in the flow of fluid brought into contact with the dermal tissues of normal animals. Changes occurring in the rate of absorption of fluid from the skin or in its passage from the blood stream under various physiological and pathological conditions will be described in the following paper. The changes that occur in the pressure of edema fluids under differing conditions and those in the resistance offered by tissues to the passage of fluids will be described in succeeding papers.

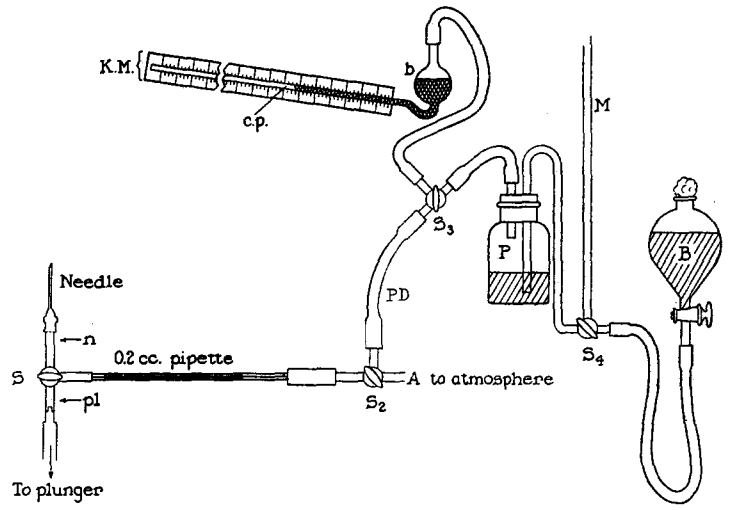
Apparatus and Methods

Reference to Text-fig. 1 will make clear the nature of the apparatus which was employed to bring fluids into contact with the dermal tissues and to measure minute amounts of fluid that were taken up by the skin. A 0.2 cc. Bureau of Standards pipette, 0.5 mm. in bore and graduated in cubic millimeters, contained the test fluid to be brought into contact with the tissues. The lumen of the pipette was layered with paraffin, but so thinly that the bore remained greater than 0.4 mm. and, as will be seen below, movement of fluid through it was not subjected to strong capillary forces like those occurring in micropipettes but remained unhampered. The tip of the pipette was ground to fit perfectly into one arm of a three-way glass stopcock, S in Text-fig. 1. The arm at right angles to this was ground to fit a gauge 30 platinum-iridium Luer needle, n, through which the test fluids were brought into contact with the tissues, as will be described below. The third arm, pl, of the cock S, being directly opposite the needle, was employed, as will also be described below, to pass a plunger into the needle from time to time to test for obstruction. All joints in the apparatus were kept air-tight and were tested in every experiment.

It was planned to bring test fluids into contact with the tissues without pressure in certain experiments but in others to subject the fluids to various known pressures. The apparatus was therefore arranged in such a way that the contents of the pipette might communicate with the atmosphere of the room or might be subjected to pressure by a device to be described below. To accomplish the first object the open end of the pipette was sealed to rubber pressure tubing with walls ½ cm. thick, connecting with one arm of another three-way stopcock, S_2 in Text-fig. 1. As indicated in the figure, this cock could be opened directly to the room atmosphere through the arm labeled A, so that the fluid in the pipette and the needle was subjected to no pressure.

When desired, pressures ranging from 0.2 mm. to 60 cm. of water were brought to bear upon the contents of the pipette and needle by a device represented schematically in Text-fig. 1 and appearing at the right of the photographs shown in Figs. 1 and 2. The device consisted of a water filled bulb, B, connected by pressure tubing with a manometer, M, and a sealed pressure bottle, P. All three were fixed on an upright brass stand in such a way that the bulb, B, could be raised or lowered by rack and pinion screws. The fluid of the bulb communicated with the sealed bottle through pressure tubing and a three-way stopcock, S_4, as shown in the text-figure. When the cock S_4 was opened and the bulb B was raised, fluid flowed from the bulb into the large-bore manometer M, which measured the pressure exerted in turn upon the air situated above the fluid in the bottle P. More pressure tubing led from the air space in the pressure bottle to a three-way stopcock, S_3, and thence to the stopcock S_2 which led either to the

pipette or to the atmosphere, as already mentioned. With cock S_3 open and cock S_2 closed to the atmosphere, the air space in the pressure bottle, P, communicated directly with that in the pipette. When very small differences in pressure were to be employed, a second manometer, KM (Text-fig. 1), was used. This consisted of a capillary tube, cp, open to the atmosphere at one end and leading to a small glass bulb at the other. To increase the sensitivity of the manometer, by decreasing the weight and viscosity of the registering fluid, kerosene colored with Scharlach R was used to partly fill the bulb and glass capillary. The bulb communicated through pressure tubing and the stopcock S_3 with both the pipette and the pressure bottle. This manometer was firmly fixed to the pressure bottle, with the tubing inclined upwards so slightly that a rise of one centimeter in the level of the water in bulb B and manometer M caused a movement of 20



TEXT-FIG. 1. Schematic diagram of the injecting device and the apparatus used to put pressure upon the fluid in the injecting device. The text-figure illustrates the principles in the construction of the manometers described in the text.

cm. on the part of the kerosene in manometer KM. The capillary tube of KM, 30 cm. in length, permitted pressure measurements to be made only when the latter were less than that exerted by a column of water 1.5 cm. in height. When higher pressures were put upon the fluid in the pipette, the manometer KM was shunted from the circuit by turning the cock S_3 appropriately.

It was obvious at the outset that the amount of fluid which would be absorbed by normal skin through a gauge 30 needle would be minute indeed. Methods were therefore devised to observe through a low-power microscope the movement of fluid in the pipette. It was further necessary to prevent all movement of the meniscus in the pipette resulting from expansion or contraction of the fluid following the slightest change in room temperature.

Accordingly, as shown in Figs. 1 and 2, the pipette, injecting needle, and stopcock *S* were mounted horizontally in a constant temperature bath and submerged beneath the water. As will be described below, it was also necessary to place the experimental animals in glass dishes partly submerged in the bath and equipped with portholes sealed with rubber tissue which allowed the submerged needle to pass into the dish for injection of the test fluid into the animal. Temperature variations of more than 0.02°C. were avoided by use of the vacuum tube method of thermal control, described by Schmitt and Schmitt (6), in which a toluol-mercury regulator is connected with the grid circuit of a thyratron tube.[1] This arrangement reduces the current passing through the regulator to such an extent that the mercury surface remains clean for long periods of time. A knife blade heater unit warmed the water, which was stirred by a propeller. In our apparatus, to avoid vibration, a two-blade propeller an inch in diameter was revolved 120 times a minute by a motor mounted apart from the water bath.

As shown in Figs. 1 and 2, the needle, stopcock (*S*), and a pipette (*P*), all submerged in the water of the bath, were supported horizontally on a brass table, one end of which, *Tb* (Fig. 1), was just enough lower than the other, *Ta*, to make room for the stopcock *S* and allow the needle and pipette to lie at the same level. To obtain brilliant illumination of the fluid meniscus within the pipette the higher part of the table, *Ta*, on which the pipette rested, was fashioned like a picture frame, with an open center under which a concave mirror was placed. This mirror focused upon the pipette the light from an arc lamp situated outside the water bath. The light from the lamp was cooled by passage through water before it entered the bath.

As shown in Figs. 1 and 2, the objectives of a binocular microscope could be moved over the graduated portion of the pipette. Protected by a glass cylinder they extended into the water of the bath, since this covered the pipette. Two ocular micrometer scales, cemented together in the eyepiece of the microscope so that the lines of one lay directly over the lines of the other, prevented errors of parallax while observing the fluid meniscus. A flow of 0.08 c.mm. moved the meniscus from one line of the ocular scale to the next. As the sharply focused meniscus itself occupied less than the width of one of the lines of the scale, one could estimate accurately and with ease the movement through one-fifth of the space between the lines. This represented a flow of 0.016 c.mm. With practice, one could estimate a meniscus movement through one-tenth of the space, corresponding to a flow of less than 0.01 c.mm. It is to be stressed here that experiments were attempted only when the meniscus was perfect. Much more will be said of this below in describing control experiments.

To bring fluid into contact with the connective tissue of living skin in such a way that it failed to enter blood or lymphatic vessels directly, the apparatus was employed as follows:

White mice were mainly used, anesthetized either with sodium luminal, as described elsewhere (3), or by intraperitoneal injection of a 1 per cent solution of nembutal, 0.5 cc. per 25 gm. of body weight. The animals were further prepared for experiment as will be described below. They were brought below the surface of the water bath so

[1] Type FG27, General Electric Company mercury vapor tube, controlling a peak current of 5 amperes by a grid in which a current of less than 0.1 milliampere may flow.

that fluid could be brought into contact with the skin through the submerged needle. To accomplish this the animal was placed in a water-tight glass dish, open at the top but partly submerged in the water bath and supported by an adjustable table, marked T_2 in Fig. 2. Portholes bored in the sides of the dishes, at various levels, and sealed with rubber tissue, permitted the needle's tip to be thrust into the dish while most of its shaft remained submerged in the water. The area of skin to receive the needle was brought exactly level with the tip of the latter by adjusting the level of the table, T_2, or by supporting the animal in various positions by molds of plasticene.

Before placing the animals in the glass dish, an area of skin of the ears, back, or thighs was prepared for the experiment in the following manner. With a dissecting needle, ground to less than 0.1 mm. in diameter, and under a binocular microscope, a tunnel parallel to the surface of the skin and 3 to 4 mm. in length was made through the tissues of the subpapillary layer of the corium. When the ear of the mouse was used this tunnel was placed midway between the large radiating vessels and was directed from the tip toward the base, parallel to the course of the lymphatics. Hence the latter were almost never injured, as will be made clear further on. Thus prepared the animal was transferred to the glass dish of the water bath and placed so that the tip of the needle, the level of the fluid in the pipette, and the skin area to be injected, all lay in the same horizontal plane. Under a second binocular microscope, not shown in the figures, and with stopcock S turned so that fluid remained imprisoned in the needle, the latter was slowly pushed into the tunnel in the skin to reach its further end, and finally adjusted in place so that there were no visible signs of tension. The shaft of the injecting needle had a much larger diameter than the dissecting needle used originally to open the pathway through the tissues, the result being that blood or lymphatic capillaries which might have been torn in making the tunnel were occluded.

Next the stopcock S was turned in such a manner that a hair-like, gauge 40 steel wire (W in Fig. 1), ground to a rounded tip, could be gently inserted into the needle like a loosely fitting plunger. With the aid of the microscope the blunt tip of the wire was used with the utmost care to push away the tissue at the needle point, thus forming a tiny cavity, with no torn blood and lymphatic capillaries in its wall. The plunger was then withdrawn slowly so that the fluid in the needle filled this new formed cavity. The amount of fluid which came in contact with the tissues beyond the cavity cannot be estimated, but it must have been relatively constant, for all the experiments were made in the same way.

Since the needle and stopcock lay below the surface of the water of the bath, the plunger was inserted through rubber and glass tubing attached to the cock, as shown in Fig. 1, at R. The plunger entered the tube through a water-tight vaccine bottle cap. The tubing was filled with Locke's solution and a side arm, ending in the barrel of a Luer syringe (L), was brought out over the edge of the water bath. Through this arm fluid could be forced into the needle if desired. If the plunger was to be passed into the needle, the syringe (L) was first lowered into the water bath until the fluid in the side arm lay at the same level as the fluid in the pipette.

During the manipulations the fluid in the pipette had been shut off from the needle. Cock S was now turned so that the needle and pipette communicated with one another again, the latter being open to the atmosphere through stopcock S_2. As result the fluid in the pipette and the needle and the tissues came to equilibrium. Thereafter all movements of the meniscus of the fluid in the pipette were continuously observed, but at any

time one could test for obstruction in the needle by turning the cock *S* appropriately, to allow the plunger to be run through the needle until its end appeared in the small cavity in the tissues. In every experiment readings were made only when the pipette remained so clean that the meniscus showed no distortion. Whenever unsymmetrical curvature of the meniscus appeared, a new experiment was begun with a fresh pipette.

In this manner, when the end of the pipette was open to the room air, the fluid in the needle and in the measuring pipette was simply brought into contact with the tissues at atmospheric pressure, and events were allowed to take their course. An observer seated at the binocular microscope watched continuously for movement of the meniscus in the pipette with respect to its position in relation to the ocular micrometer scale. The amount of movement, if any, was recorded at ½ minute or 1 minute intervals. The success of the experiments depended wholly upon placing of the needle in such a way that no fluid could pass directly to or from torn lymphatics or blood vessels.

Several sorts of controls to the conditions were employed. If red blood cells appeared in the tissues, or if a distortion of them visible under the microscope resulted, the experiment was abandoned. In more than a hundred experiments we brought into contact with the dermal tissues, in this manner, solutions sufficiently colored to be visible in blood capillaries and lymphatics. These fluids entered the lymphatics in less than 4 per cent of the trials and appeared in the blood vessels in only one instance.

To test for blood vessel injury a vital dye, pontamine sky blue, was employed in scores of tests. This dye has been used in many of our previous studies on lymphatics (1–5, 7–12), and its preparation has been described (3). At the end of each experiment, while the needle remained in the skin, an aqueous isotonic solution of the dye (21.6 per cent) was mixed with Locke's solution to give a final dilution of 5.4 per cent, and 0.1 cc. was injected intravenously into the tail of the animal. The dye, which escapes slowly from the circulation (13–15), could be seen in a few seconds filling the larger blood vessels and capillaries of the ear. Ecchymoses of dye signifying rupture of capillaries were almost never seen; on the few occasions when they did appear the experiment was ruled out. In all of these trials dye circulated with the blood in the smaller vessels and capillaries at the very edge of the cavity which had been formed at the tip of the needle. Dye-colored blood could even be seen flowing through capillaries that lay between the bevel of the needle and the surface of the skin. The capillaries in the remainder of the ear also appeared like those in normal tissue and like those in the animal's intact other ear (Figs. 3 to 5).

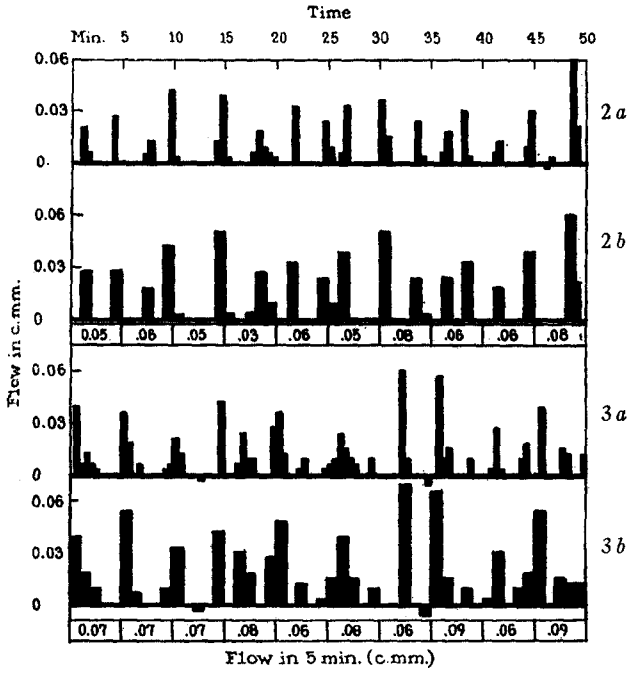
Fig. 3 (× 12) shows the ear of an 18 gm. mouse half an hour after placing the injecting needle in the subpapillary layer of the skin. During this period, as well as at the moment the photograph was taken, Locke's solution was passing through the needle into the tissues at atmospheric pressure. 0.1 cc. of the dye solution was then injected into the animal's tail vein. Fig. 4, taken 4 minutes later, shows the dye in the capillaries round about the needle. As judged by the speed of coloration in the vessels, the rate of blood flow close to the needle was similar to that in other parts of the ear. No dye

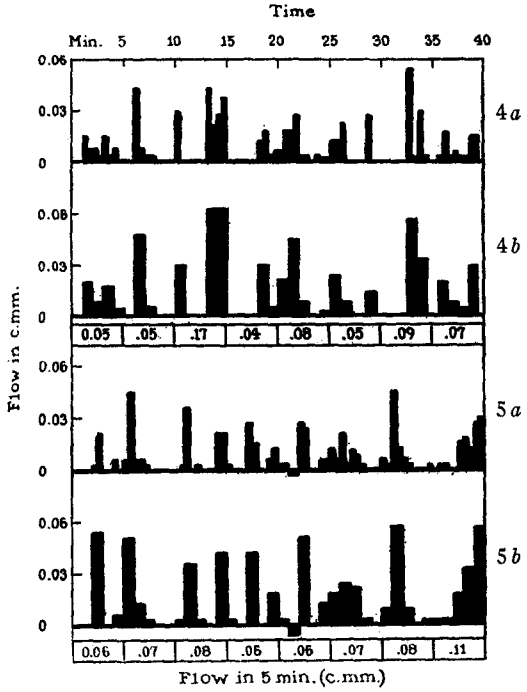
ecchymoses occurred such as would promptly have formed had any blood vessels been torn. Figs. 5 *a* and 5 *b*, magnified only six times, compare the amputated ears of a mouse after a similar experiment. For 40 minutes Tyrode's solution at atmospheric pressure had been in contact with the tissues of the skin of the left ear (Fig. 5 *a*) through a needle placed in the tissues at the situation indicated by the broken lines. 4 minutes after an intravenous injection of dye the needle was withdrawn. Both ears were amputated at once and photographed 2 minutes later. The white area at the ear edge, below the position of the needle, shows the spot at which the ear was grasped with forceps to hold it while the needle was put in place. The vessels in this region, which had been squeezed, remained occluded and cannot be seen because no dye entered them. It is to be noted that the tissue about the region in which the needle lay (Fig. 5 *a*) is no more highly colored than other parts of the ear. This fact tells much concerning the local conditions. It has been shown repeatedly in this laboratory, and by the work of others, that intravascular dye escapes more rapidly into injured than into normal tissue (7, 8, 13–15). Had the vessels near the needle tip been injured, a marked intensification of the color about the needle would have resulted.

The Movements of Fluid into Cutaneous Tissues at Atmospheric Pressure

In more than four hundred tests made in the manner just described we have brought Locke's or Tyrode's solution into contact with the tissues of the skin of the ears, backs, and thighs of anesthetized mice or rabbits at atmospheric pressure, that is to say simply allowing matters to take their course while the pipette remained open to the room air. Under these circumstances the solutions were taken into the tissues very slowly and with a surprising intermittency. Short periods in which fluid was taken into the skin were rapidly succeeded by longer periods in which no fluid entered it or, rarely, by backflow from the tissues to the apparatus. An observer continuously watching the fluid meniscus in the pipette saw no movement for periods varying from 1 to 5 minutes or even more. Then, suddenly the meniscus bulged backward for a second or two, a distance too small to measure, and then almost immediately moved in the opposite direction toward the tissues, as inflow began. Some of the periods of inflow were brief, lasting only 10 to 15 seconds but many endured for a minute or more and a few for several minutes. Flow usually ceased as abruptly as it began and there followed a period of no movement. As will appear below in the description of control experiments which tested the character of equally slow flow through the apparatus alone, no such intermittency took place.

In Text-figs. 2 to 5, inclusive, we have charted the inflow of fluid into the skin in four experiments typical of all. The heavy base line in these and the other charts indicates that the observations were continuous. It





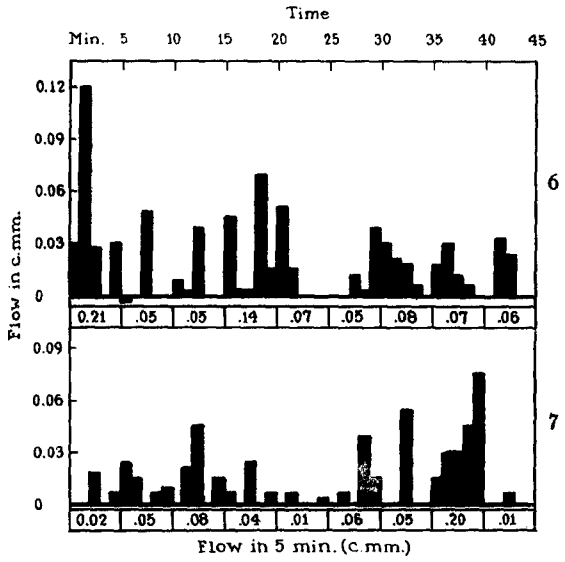
TEXT-FIGS. 2 to 5, inclusive. The intermittent entrance of Locke's solution, at atmospheric pressure, into living skin. The readings have been plotted for each experiment at 30 second intervals (Text-figs. 2*a*, 3*a*, 4*a*, and 5*a*) and at 1 minute intervals (Text-figs. 2*b*, 3*b*, 4*b*, and 5*b*). See text for interpretation.

will be seen that in the present instances inflow was intermittent. Figs. 2*a* to 5*a* show the amounts of inflow which took place during each ½ minute interval during these experiments, and Figs. 2*b* to 5*b* the flow, in the same experiments, when recorded at 1 minute intervals. The latter figures have been included mainly to furnish a comparison with data presented in later papers and obtained from experiments in which we were unable to make readings at intervals shorter than 1 minute. In all the text-figures, each of the solid black columns standing above the base line represents the amount of inflow during that particular ½ minute or 1 minute period. Columns extending below the line depict backflow. All should be compared with the following charts which demonstrate the characteristics of fluid flow through the same apparatus and needle during the control experiments to be described below.

As already stated, an observer watched continuously the movement of the meniscus in the pipette and recorded the amount of movement observed at the end of each 30 second or 1 minute period. It is to be stressed, therefore, that the columns in the figures represent merely these recordings, that is to say, the amount of fluid that had entered the tissues at the end of a 30 second or 1 minute period. The columns give only the roughest indication of the curve of the changing rates of flow and of precisely when it began and stopped. One could only measure accurately the position to which the meniscus had moved at the end of half a minute or 1 minute. Nevertheless on many occasions the movement of fluid was sufficiently great to show that the beginning of flow and its cessation were abrupt. For example, in Text-fig. 2*a* the second column from the left side of the figure shows that in the 30 second period commencing 4 minutes after readings were begun and ending a half minute later, an inflow of a little less than 0.03 c.mm. took place. The flow recorded here actually began at about 4 minutes and 10 seconds and endured but 15 seconds, so that during half of this 30 second period there was no flow. The column in Text-fig. 2*b* immediately below shows the same amount of flow occurring in the 1 minute period between the 4th and 5th minutes of the experiment because there was no further flow in the last 30 seconds of this period. Again, as shown in Text-fig. 2*a*, during the 47th minute of this experiment there was a minute backflow in the first 30 seconds and an equal inflow in the second 30 seconds, but in Fig. 2*b*, which shows the changes in flow as read at minute intervals, the effects have canceled each other and no flow is recorded. By contrast, the movement was not always sudden or abrupt. In Text-fig. 3*a*, 24½ minutes after beginning this experiment there was some slight movement of fluid in seven consecutive half minute periods. This movement was steady, increased for four periods, and then decreased in three.

The charts show that by and large the periods of fluid entrance were brief in relation to the periods of no movement; they were shorter, as mentioned above, than the black columns would indicate and the periods of stasis were correspondingly longer.

The take-up of fluid during each 5 minute period in every experiment is given in all of the text-figures. Although in a few instances as much as 0.1 to 0.12 c.mm. entered the tissues per 5 minutes and in others, as will be detailed below, there was no flow, in most of the animal experiments the



TEXT-FIGS. 6 and 7. The intermittent interstitial movement of Locke's solution forced into living skin by pressures of 2.0 and 1.0 cm. of water, respectively. Readings from two different experiments are shown, at minute intervals only.

rate of inflow at atmospheric pressure was surprisingly constant, in more than 80 per cent varying only from 0.04 to 0.08 c.mm. per 5 minutes.

Intermittent inflow did not appear in every instance. In about 3 per cent of our trials a continuous, irregular inflow occurred at about the same rate as in the other animal experiments but without evidence of periodicity. The irregularities which appeared were like those observed in the controls, to be discussed below. In another 3 per cent of our experiments there was no flow at atmospheric pressure. In such instances the pressure of a column of water 1 or 2 cm. in height, applied, as described above, to the contents of the pipette, usually produced flow. If none occurred, the stopcock *S* was closed and the plunger inserted to test for obstruction. All instances showing it were ruled out.

In the experiments so far considered no edema of the skin was visible under the microscope but in many instances, 20 to 30 minutes after placing the needle in the skin, edema appeared. In these instances the intermittent inflow of fluid halted and intermittent backward movement began from the tissues into the apparatus, as will be described in the following paper.

Fluid brought into contact with the connective tissue of normal skin and then forced into it under pressure continues to enter it in a periodic manner at all pressures less than 4 to 4.5 cm. of water. Text-figs. 6 and 7 show the variations of flow into the skin of the mouse ear, as observed at 1 minute intervals when pressures of 2 and 1 cm. of water, respectively, were brought to bear upon the fluid introduced by way of the needle. As the figures show, the periods of no movement and of abrupt flow still made their appearance.

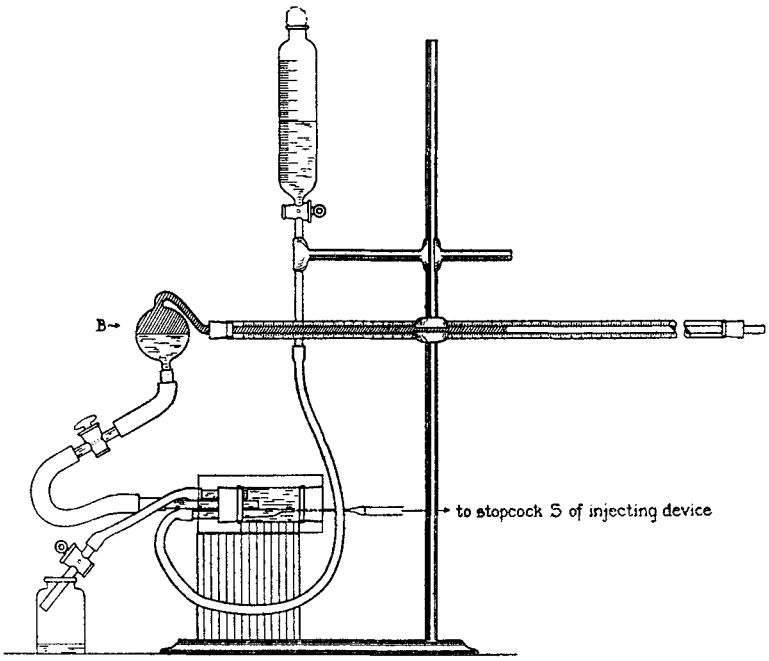
The charts presented here will serve as a base line for comparisons with other experiments, to be described in later papers, in which the effects of both low and high pressures upon the interstitial movements of fluids will be discussed. Of these it will suffice to say here that pressures slightly higher than 4.5 to 5.0 cm. of water result in the continuous entrance of some fluid and further increases abolish the intermittent character of the inflow.

CONTROL EXPERIMENTS

How is the intermittency of flow to be interpreted? Is it produced by physiological happenings in the tissues of the animals or is it an artifact caused by the apparatus? As already described and as shown in the text-figures, the amount of flow in the animal experiments was extremely small. Adhesion of the moving fluid to the paraffined pipette, even if too slight to distort the meniscus, must have influenced its apparent motion, yielding slower movement or none when the meniscus adhered to the pipette, and faster apparent motion when the tension in the fluid became great enough to break the adherence. It seemed imperative to determine whether or not there might be irregularities of the movement of the meniscus when fluid passed through the apparatus alone, which could account for the intermittent movement we observed in the animal experiments.

More than eighty control tests were made in which fluid was passed through the apparatus alone at various rates of flow, faster, slower, or equal to that of the observed flow into the skin of the animals. In none of these control tests did the characteristic intermittent movement occur that was seen in animal experiments. Other, but quite different, irregularities of flow, produced no doubt by surface forces about the meniscus, did appear. These, and the control experiments demonstrating them (Text-figs. 9 to 14, inclusive), will now be discussed.

In the animal experiments the needle lay in a pool of fluid within the tissues and the Locke's or Tyrode's solution was drawn through the pipette and needle by forces of an unknown nature within the tissues. Comparable control experiments were devised in which fluid was drawn through the apparatus by osmotic forces acting at the tip of the needle. To obtain flow through the apparatus which would be as slow as that occurring in the



TEXT-FIG. 8. Schematic diagram of the apparatus employed in some of the control experiments and described in the text. In this figure, to illustrate the principles of the apparatus, the manometer has been drawn above the chamber which contains the Locke's solution. Actually both were at the same level so that there would be no pressure differences between them.

animal experiments the needle and pipette were filled with Locke's solution and the needle's tip submerged in it. Through a capillary pipette saturated sodium chloride solution was introduced into the Locke's solution close to the needle's tip. The higher concentration of salt near the tip of the needle drew out fluid through it by osmosis at a rate which approximated that observed in the animal experiments.

As indicated by the diagram in Text-fig. 8, the needle connected to the pipette of the injecting device was thrust through the stopper of a glass cylinder filled with Locke's solution. Three tubes entered the other end of the cylinder. The first, a capillary

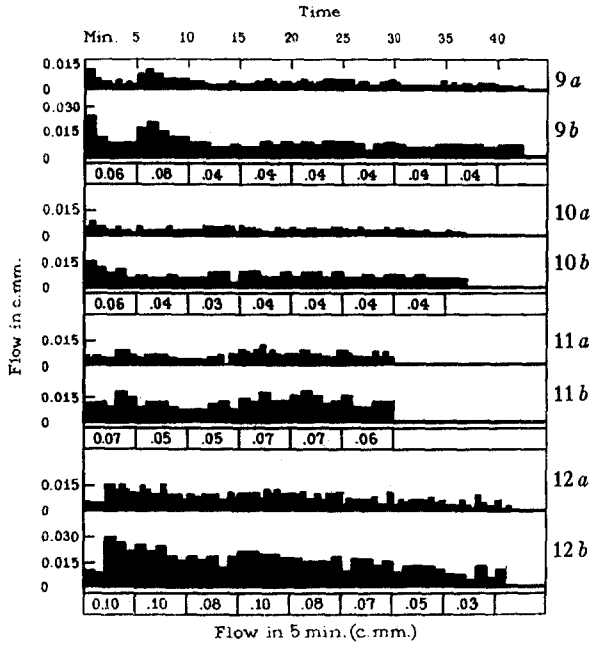
tube situated close to the needle's tip contained saturated sodium chloride solution and led to a burette filled with the same fluid. The second led to an overflow pipe and the third to a sensitive manometer. The latter, designed to measure minute pressure changes in the chamber, consisted of a bulb (*B*, Text-fig. 8) connected with a horizontal capillary tube, 0.1 mm. in bore, $1\frac{1}{2}$ meters in length, backed by a millimeter scale and open to the atmosphere at its further end. The bulb was half filled with the Locke's solution, which also completely filled the glass cylinder and needle and partly filled the pipette, the end of which was left open to the atmosphere. To increase the sensitivity of the manometer, colored kerosene was layered above the Locke's solution in the bulb until it completely filled the latter and extended into the capillary. We have drawn the manometer above the cylinder in Text-fig. 8, to indicate the nature of the apparatus. Actually the two were placed at the same level so that no pressure was exerted between them. An increase in the pressure in the cylinder, equivalent to that caused by a column of water only a fraction of a millimeter in height, resulted in movement of the colored kerosene for several centimeters along the scale. The kerosene meniscus could be brought back to its original position by opening the overflow tube until the initial pressure was restored.

The tests were carried out in the following way. The Locke's solution in the glass cylinder, needle, and pipette was allowed to come to equilibrium by opening the stopcock on the overflow tube. It was then closed. To induce extremely slow flow in the pipette of the injection apparatus by osmosis, 0.10 cc. of the saturated NaCl solution was allowed to flow slowly into the cylinder after we had observed the position of the kerosene meniscus and had closed stopcock *S* between the injecting needle and the pipette. As the maneuver momentarily increased the pressure in the cylinder, the stopcock on the overflow valve was at once released until the kerosene meniscus returned to its original position, indicating that the pressure of the fluid in the cylinder and that of the fluid in the pipette was again equal, although separated from each other by the closed stopcock *S*. The cock on the overflow tube was next closed and cock *S* opened, allowing the Locke's solution in the pipette to communicate with that in the needle and chamber.

The presence of strong NaCl solution close to the needle's tip produced, by osmosis, a movement of Locke's solution in the pipette of the injection device. As the concentration of NaCl near the point of the needle became less, the rate of flow slowly decreased and finally stopped. By adding the proper amount of salt solution to the chamber, about 0.10 cc., we induced in the injecting device a flow of Locke's solution which was at first more rapid than the usual take-up of fluid by living skin. As the experiments proceeded the rate of flow became equal to and finally less than that observed in the animal experiments. If during the test any change in pressure occurred within the cylinder, the kerosene meniscus, indicating the change, was brought back to its original position by withdrawing or adding minute amounts of fluid to the cylinder through the overflow tube.

The data from several typical trials which endured from 30 to 40 minutes have been plotted in Text-figs. 9*a* to 12*a*, respectively, showing the movement of the meniscus in the injecting device in each half minute period. In Text-figs. 9*b* to 12*b*, the movement has been plotted at minute intervals. In all these figures and in all the remainder, the flow during each 5 minute period is given below the drawing. The charts show the characteristics of the movement of the fluid meniscus in the apparatus when flow is

exceedingly slow (Text-fig. 10*a* and *b*) and also when it is more rapid (Text-fig. 12*a* and *b*). In all instances, although it showed certain irregularities, flow was continuous. As already suggested, these irregularities were probably caused by clinging of the meniscus to the sides of the pipette or perhaps to the inertia of the column of fluid. The experiments are typical of scores of others done in the same manner.

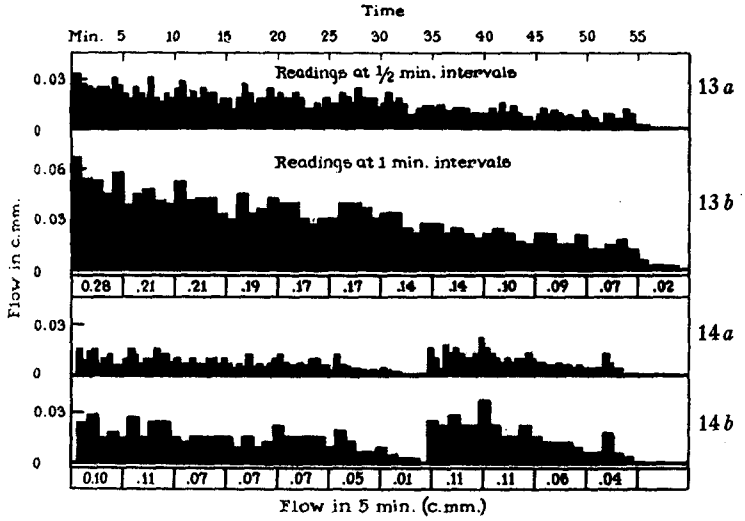


TEXT-FIGS. 9 to 12, inclusive. Control experiments. Text-figs. 9 to 12, inclusive, record the flow of Locke's solution through the pipette and needle of the injecting device during control experiments in which the osmotic forces in the "salt chamber" were used, as described in the text, to draw fluid through the injecting device. Text-figs. 9*a* to 12*a* show the movement of the meniscus plotted at ½ minute intervals. In Text-figs. 9*b* to 12*b* we have plotted the movement at 1 minute intervals. The slight irregularities as plotted contrast strongly with those shown in Text-figs. 2 to 7, which were obtained in animal experiments.

We have shown above that Locke's solution forced into the skin at pressures of 4.5 cm. of water or lower continues to enter the tissues intermittently. In many other experiments, to be described in later papers, fluids were forced into the tissues by the application of pressure to the contents of the injecting pipette, employing for the purpose the pressure bulb and manometer already described (Text-fig. 1 and Figs. 1 and 2). It seemed possible that under these conditions in which fluid was no longer drawn into tissues by forces acting at the needle's tip but was forced into them by pressure, applied directly to the fluid in the injecting apparatus, there might

be a difference in the character of the movement of the meniscus. Suitable controls for these experiments were obtained by applying so little pressure to the contents of the pipette that fluid moved through it at the same rate at which, in the animal experiments, it had been drawn into the skin by forces within the tissues themselves.

In eight experiments a large glass dish was filled with Locke's solution until the latter stood above the brim without overflowing, while the needle of the injection apparatus was submerged horizontally just beneath its surface. After the fluid in the pipette had been brought to equilibrium with the atmosphere, a pressure of about 0.2



TEXT-FIGS. 13 and 14. Text-figs. 13 and 14 represent the results of control experiments, showing the movement of Locke's solution through the apparatus when forced by very slight pressure. The movement, depicted at 30 second and 1 minute intervals, respectively, was continuous, as in the preceding records. See text.

mm. of water, as registered by a movement of 4 cm. by the kerosene meniscus *KM* (Text-fig. 1), was put upon the pipette from the leveling bulb. This pressure forced fluid from the pipette into the relatively large "lake" of Locke's solution in the dish. As flow continued, it became slower. Text-figs. 13*a*, 13*b*, 14*a*, and 14*b*, from two typical experiments, show the readings of the flow of fluid through the pipette at ½ minute and 1 minute intervals. In all a continuous movement of the meniscus occurred, accompanied by the irregularities observed in the other control experiments. In one of these trials (Text-fig. 14*a* and *b*), after flow had almost ceased, pressure was increased again and the flow resumed. The rate of flow at the beginning of these control experiments was faster than the flow at atmospheric pressure into the skin of normal mice (Text-figs. 2 to 5). At the end of the control tests flow was slower. Whether fast or slow, it was relatively uniform. In many other control experiments, greater pressure yielded faster flow and the movement of the meniscus became still more uniform.

The control experiments show further that the pressure required to move Locke's solution through the apparatus at the rate of flow encountered in animal experiments is negligible. It is to be noted that both the animal experiments and the controls were made in such a way that the needle's tip lay submerged in fluid, thereby avoiding surface tension effects from drop formation at the needle's point.

That the intermittent flow observed during the animal experiments cannot have been caused by clinging of the meniscus to the wall of the pipette is shown too by certain happenings during the course of these tests. In many of the animal experiments the pipettes became slightly dirty. Under these circumstances flow often stopped temporarily but as fluid continued to be drawn into the skin the tension distorted the meniscus, changing its contour as part of it stuck to the pipette wall. By contrast, fluid flowing into the tissues from a perfectly clean pipette stopped and resumed movement with no change in the shape of the meniscus. Even when a pressure of 1 or 2 cm. of water was put upon the fluid in the pipette the intermittent flow and stop still continued, Text-figs. 6 and 7. In all our control experiments such pressures kept the meniscus moving at all times. A cessation of flow against a pressure of 1 or 2 cm. of water never occurred, even in a dirty pipette, when fluid moved through the apparatus alone.

COMMENT

Intermittent flow through the apparatus occurred only in the animal experiments, not in the controls, and hence one may conclude that some physiological happening is responsible. The phenomenon is not to be explained by physical forces active in the pipette.

Fluid placed interstitially must either be absorbed periodically from local regions of tissue, or changes must occur which produce an intermittent movement of interstitial fluid. This matter will be dealt with in the following paper.

SUMMARY

Methods have been devised to bring microscopic amounts of fluid into contact with cutaneous connective tissue, under pressure or without pressure, in such a manner that it enters neither blood capillaries nor lymphatics directly. The take-up of fluid brought into contact with the tissues in this way has been measured and its characteristics studied. Elaborate control tests, here described and discussed, have indicated the possible errors in the employment of these methods.

Locke's or Tyrode's solutions brought into contact with the cutaneous tissues, by the method described and at atmospheric pressure, pass into the tissue intermittently. Forced into the skin by pressures of 1.0 to 2.0 cm. of water the take-up is still intermittent in character. From this it follows that either the absorption of interstitial fluid from localized regions is periodic or the movements of interstitial fluid are influenced by intermittent physiological changes.

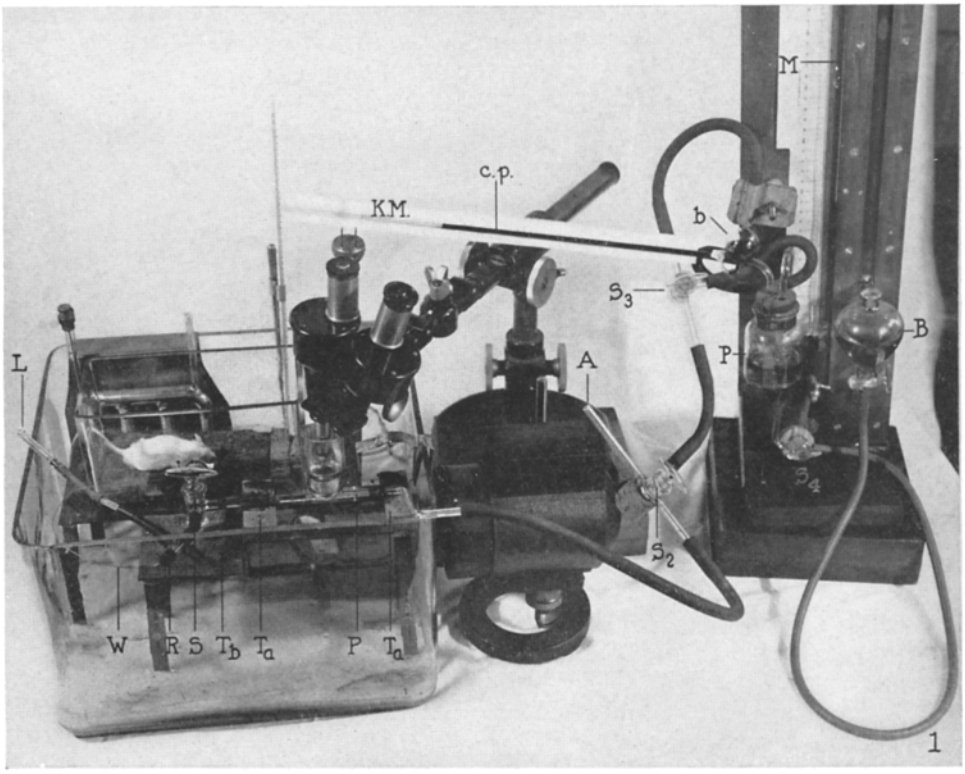
BIBLIOGRAPHY

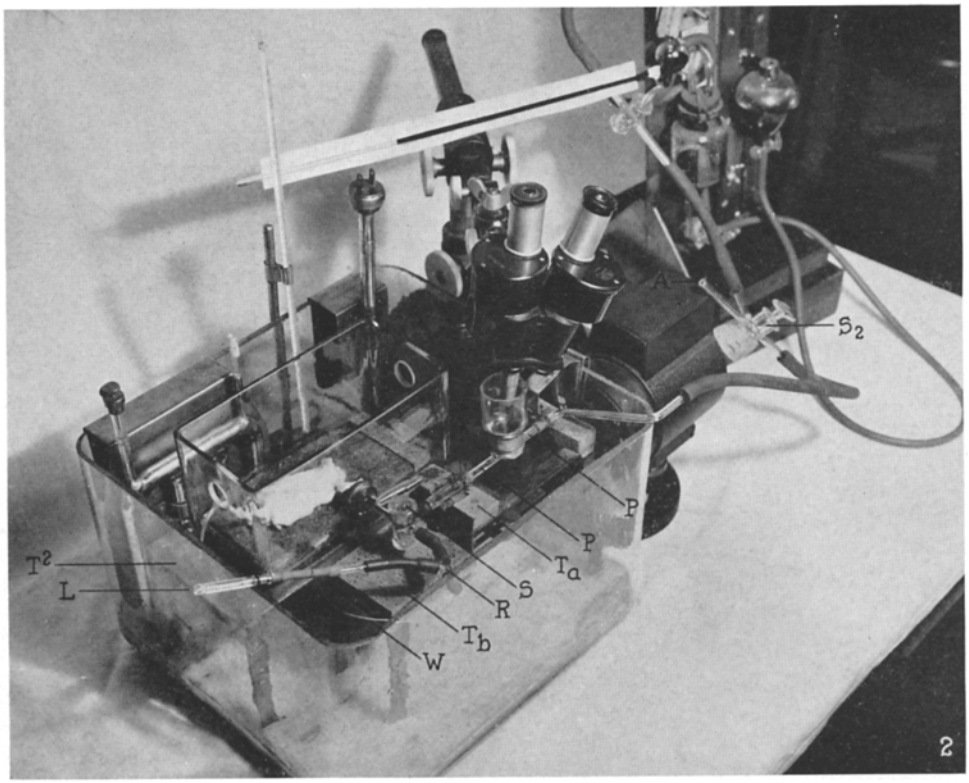
1. Parsons, R. J., and McMaster, P. D., *J. Exp. Med.*, 1938, **68,** 353.
2. McMaster, P. D., and Parsons, R. J., *J. Exp. Med.*, 1938, **68,** 377.
3. Parsons, R. J., and McMaster, P. D., *J. Exp. Med.*, 1938, **68,** 869.
4. McMaster, P. D., and Parsons, R. J., *J. Exp. Med.*, 1939, **69,** 247.
5. McMaster, P. D., and Parsons, R. J., *J. Exp. Med.*, 1939, **69,** 265.
6. Schmitt, F. O., and Schmitt, O. H. A., *Science*, 1931, **73,** 289.
7. Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1932, **56,** 223.
8. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **56,** 239.
9. Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57,** 751.
10. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1934, **60,** 479.
11. McMaster, P. D., *J. Exp. Med.*, 1937, **65,** 347.
12. McMaster, P. D., *J. Exp. Med.*, 1937, **65,** 373.
13. McMaster, P. D., Hudack, S. S., and Rous, P., *J. Exp. Med.*, 1932, **55,** 203.
14. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **55,** 417.
15. Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1932, **55,** 431.

EXPLANATION OF PLATES

PLATE 1

FIGS. 1 and 2. Photographs of the water bath, the injecting device, and the apparatus employed to apply pressure to the fluid in the injecting device. The figures are fully described in the text. To simplify the pictures, the motor, the propeller which stirred the bath, the thyratron tube, and the wiring have been removed. To avoid vibration, the propeller was held in the bath by a separate stand.





Photographed by Joseph B. Haulenbeek

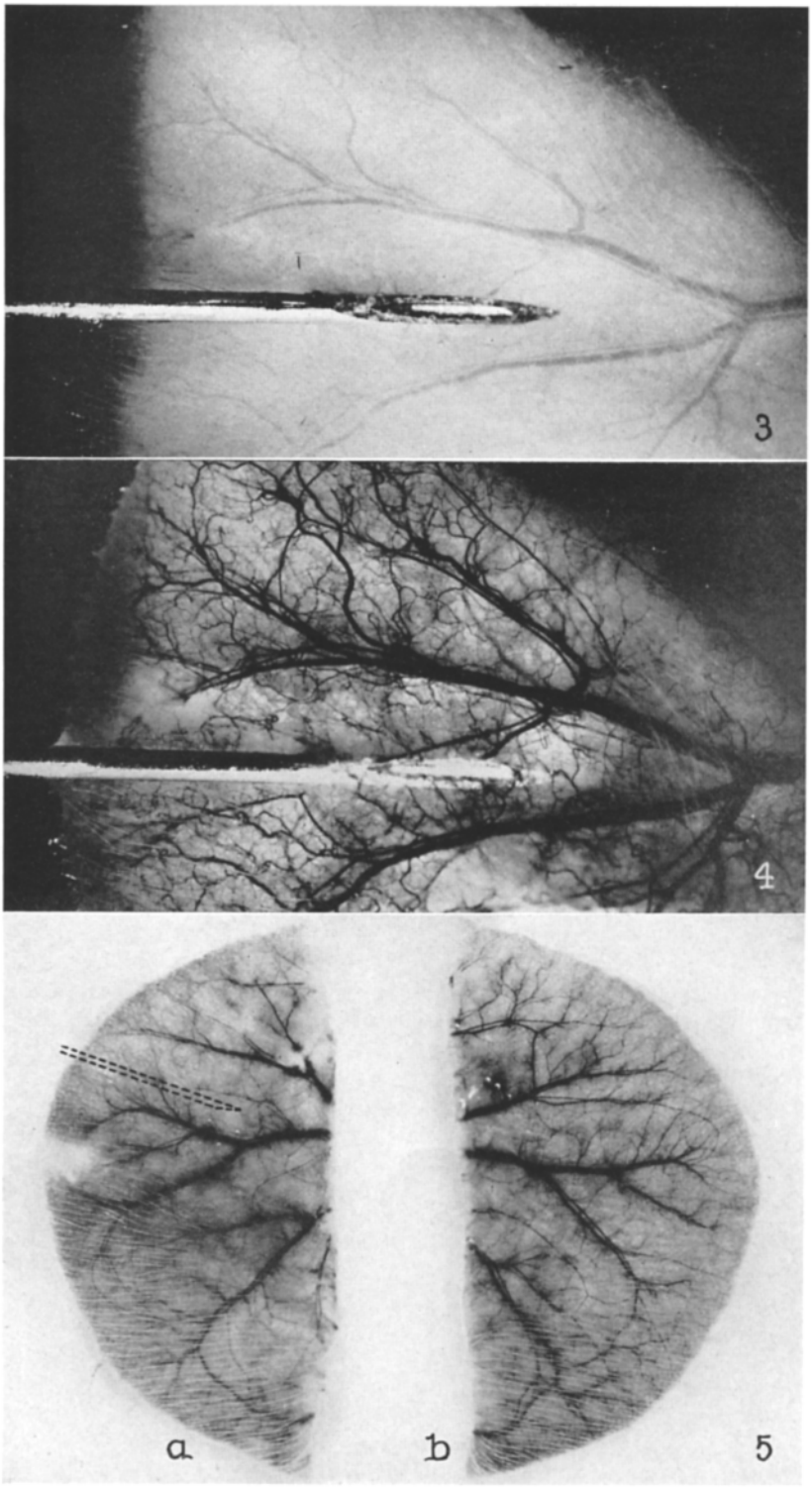
(McMaster: Intermittent take-up of fluid from cutaneous tissue)

PLATE 2

FIG. 3. A gauge 30 platinum-iridium hypodermic needle inserted interstitially into a tunnel in the skin of the ear of a living mouse, as described in the text. The needle had been in place for half an hour. It is placed very superficially and there are no signs of injury about it.

FIG. 4. The same ear about 4 minutes later, following an intravenous injection of dye to test for the presence of injured blood vessels. Note that dye has been distributed to the blood vessels next to the needle, showing that circulation is intact in these regions. There are no dark ecchymoses of dye such as form when it escapes from injured vessels.

FIGS. 5*a* and 5*b*. A comparison of the ears of a mouse, amputated 4 minutes after an intravenous injection of dye, as described in the text. For 40 minutes prior to the injection of dye, Locke's solution had been in contact with the dermal tissues of the left ear, by way of a needle introduced as shown (Fig. 5*a*). The left ear shows very little more color than the right (Fig. 5*b*). Gross injury to the left ear would have greatly increased the color. The blood vessels of both ears show approximately the same state of affairs.



Photographed by Joseph B. Haulenbeek

(McMaster: Intermittent take-up of fluid from cutaneous tissue)