FACTORS INFLUENCING THE INTERMITTENT PASSAGE OF LOCKE'S SOLUTION INTO LIVING SKIN

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As reported in the preceding paper (1), Locke's or Tyrode's solution, brought into contact with the tissues of living skin, at atmospheric pressure, in such a way that it enters neither blood nor lymphatic vessels directly, passes into the skin intermittently. The phenomenon, if understood, should throw some light upon the mechanisms of the movement of intercellular fluid and the formation of lymph. Two possibilities suggest themselves. The intermittent character of fluid entrance into the skin may be due to recurring alterations in the vascular or tissue conditions determining absorption, or there may be an intermittent expansion and contraction of tissue elements which allow fluids to move through the tissues in an irregular manner. If fluid is absorbed into the blood intermittently under natural conditions, the present concepts of the mechanics of fluid exchange require modification. If periodic changes affecting absorption take place in the tissues, then it becomes a matter of interest to know what these changes are. This paper deals with the problem presented.

Methods

The method by which Locke's or Tyrode's solution can be brought into contact with the dermal tissue of the ears, back, and thighs of anesthetized mice or rabbits has been fully described in the preceding paper (1), as have also the controls on its validity. It will be recalled that the fluids were brought to the tissue through a No. 30 gauge platinum needle with an internal diameter of 0.16 mm. The amount of tissue with which the fluid came in contact at the end of the needle cannot be determined, but must have been relatively constant, for all the experiments were done in the same way. The inflow through the fine-bore needle was extremely small but it was sufficient to permit measurements at half minute or one minute intervals. The difficulties and errors in measuring the flow of these small amounts of fluid and the precautions taken to obtain accuracy have already been described.

In the present work the method has been employed to throw light on the mechanism of absorption. Locke's or Tyrode's solution has been brought into contact with the dermal tissues of anesthetized mice at atmospheric pressure and the resulting entrance of fluid into the tissues, which occurred in 97 per cent of more than 300 experiments, has been observed and measured. In more than 100 additional experiments edema-

forming or relatively unabsorbable fluids, at atmospheric pressure or slightly above it, have been brought into contact with the connective tissue of the skin in the same manner. In many of these experiments backflow occurred into the apparatus, as will be described below, and sometimes when this happened tissue fluid clotted in the needle and obstructed all further movement of fluid. Distortion of the meniscus in the pipette often appeared. All such experiments were discarded.

The experiments lasted for varying periods, from 30 minutes to an hour usually, but often were continued longer when the skin remained normal in appearance under the microscope or when the aim was to produce pathological states. To ensure avoiding the latter short periods were in many cases employed. In all the experiments a slight hyperemia developed in the skin after placing the needle, but in most of them it soon subsided. As time went on edema of the skin, perceptible under the binocular microscope, often occurred. The findings in edematous skin will be separately discussed. At the end of many of the experiments, 0.05 to 0.1 cc. of a 5.4 or a 10.8 per cent solution of a vital dye, pontamine sky blue, rendered isotonic as already described (2,3), was injected intravenously through a tail vein. The dye promptly appeared in the capillaries about the needle's tip, and whenever blue ecchymoses formed, indicating rupture of a vessel, the experiment was discarded. This occurred so rarely that in our later experiments the dye was employed only in instances that had yielded atypical findings.

In more than 200 experiments, performed later for a different purpose, dilute dye solution was often brought into contact with the tissues interstitially, to test whether lymphatics had been torn open when the tunnel was made in the connective tissue for insertion of the needle. In only 4 per cent did dye enter a lymphatic capillary. It follows that the incidence of lymphatic injection was too low to influence the findings.

Before we could attempt to learn whether the intermittent character of the entrance of fluid into the skin is caused by a recurrent absorption into the blood and lymph or is brought about by expansion and contraction of tissue elements or blood vessels, it was clearly necessary to show that the methods employed were sensitive enough to demonstrate changes in the absorption of interstitial fluid. To accomplish this a variety of experiments were undertaken. In each a test fluid, at atmospheric pressure, was brought into contact with the dermal tissues of normal mice, and after the rate and characteristics of its inflow had been observed or its failure to enter had been noted, the conditions were experimentally altered. In the first experiments fluids of one sort or another were injected into a tail vein, while the test fluid was still flowing into the ear. It was noticed that manipulation of the tail or the insertion of a needle into the skin was followed by an immediate and brief entrance of fluid into the ear, lasting but a few seconds. As a result of this finding, it became necessary to determine the effects of pain and touch stimuli, especially those from the injecting needle itself, before satisfactory experiments could be done to determine whether or not the methods employed were sensitive enough to detect the absorption of interstitial fluid.

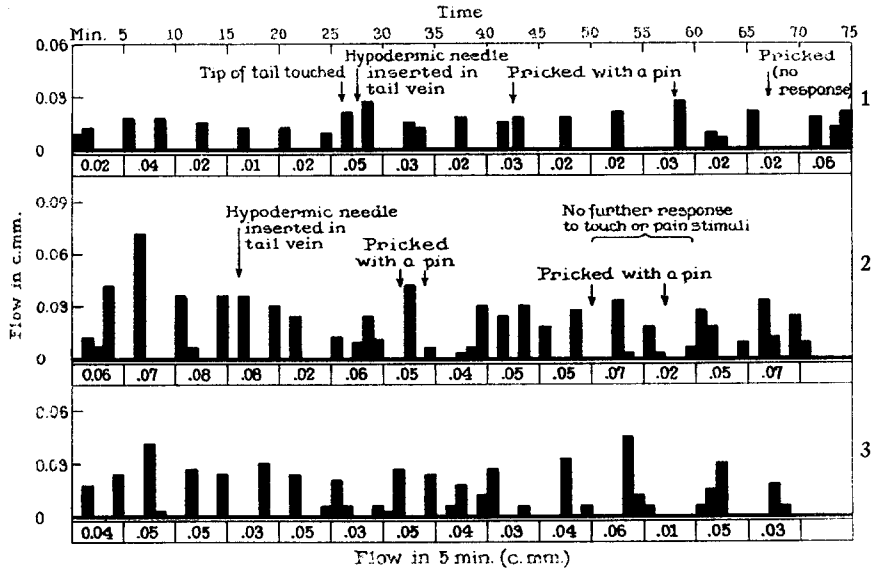
The Effects of Pain and of Tactile Stimuli

The intermittent entrance of Locke's solution at atmospheric pressure into the ears of 16 mice anesthetized with luminal (1, 2) was observed and measured for half an hour. In 12 of these the flow was found to occur in the usual manner; that is to say, brief periods of take-up lasting a few seconds were followed by periods of stasis lasting several minutes. At the end of the half hour of observation the tail was picked up and squeezed between thumb and finger or pricked with a hypodermic needle inserted into a tail vein as if an injection were about to be made. For this maneuver a moment was selected a few seconds after the end of a period of inflow, when further flow would not ordinarily be expected for 2 or 3 minutes. In each instance the manipulation or the pricking was followed in 1 to 4 seconds by brief inflow into the ear, lasting usually but 10 to 15 seconds. Following this response, with the needle motionless in the tail vein, intermittent flow into the ear was resumed in the way noted before the stimulus was given. Repetition of the stimulus a few seconds after one of the characteristic brief periods of inflow had ceased always brought about an immediate recurrence of it without the characteristic period of several minutes of no movement. After four or five repetitions of the stimuli no further responses occurred.

Text-fig. 1 shows the findings of a typical experiment. In this figure, and in all the others, the flow of fluid into the skin has been indicated as in the preceding paper. The total inflow recorded during each minute, or occasionally each half minute, of the experiment is plotted as a single black column, above a heavy black line (which indicates that the observations were continuous). If no flow occurred during any particular minute no black column appears. The columns, as mentioned in the preceding paper, do not indicate the duration of the periods of inflow, but simply the amount of fluid which had entered the skin by the end of each minute. The amount of fluid taken up by the skin was so small that one could record neither the exact moment at which fluid began to move in the pipette nor the moment at which its movement ceased. One could only measure accurately the distance the meniscus had moved in the pipette of the injecting device during a half or one minute period. Nevertheless one could estimate the periods of flow approximately. They were brief in relation to the periods of no movement, flow lasting about 15 to 45 seconds and the periods of no flow enduring several minutes. As result the gaps between the columns in the figures give a fair estimate of the relative length of time, each period of stasis being actually a little longer than the chart would indicate.

In this experiment, Text-fig. 1, Locke's solution was drawn intermittently for 26 minutes into the skin of the ear of a 29 gm. mouse. The amount of fluid entering the skin during each 5 minute period is indicated in the figure. The flow occurred at almost regular intervals, none entering the ear between whiles. The periods of inflow, which were brief, lasting less than a minute, alternated with longer periods of stasis,

enduring about 3 minutes each. During the 25th minute a characteristic, momentary inflow occurred, and then, one minute later (at the beginning of the 27th minute, as shown by the first arrow in the figure), the tip of the tail was picked up between the thumb and finger and gently squeezed with the thumb nail. At once fluid moved into the ear for a few seconds. The duration of the flow could not be determined exactly but the amount which entered during this minute, the 27th, is plotted in the figure. It is to be noted that flow took place at once after a period of no movement lasting only a minute. Presumably without the stimulus flow would not have occurred so soon. During the next minute, the 28th, a hypodermic needle filled with Locke's solution and



TEXT-FIGS. 1 and 2. The influence of tactile and pain stimuli to increase the intermittent entrance of Locke's solution into the skin.

TEXT-FIG. 3. The entrance of Locke's solution into the skin of the ear of an anesthetized mouse is still intermittent despite the blockade, by novocaine, of pain and tactile stimuli from the skin surrounding the injecting needle.

attached to a syringe was thrust into the tip of the tail and allowed to remain there during the rest of the experiment. No injection was attempted at this time and there was no movement of the ear perceptible under the binocular microscope. As shown by the second arrow in the text-figure, the stimulus brought on a period of flow immediately. The entrance of fluid into the ear for the 5 minute period in which these manipulations were made was greater than that occurring previously but for the next 10 minutes, although the needle remained in the tail, only as much flow took place as prior to the increase. During the 42nd minute one of the periods of fluid entrance took place. As soon as the meniscus in the injecting apparatus ceased to move, showing that flow had stopped, the tail was pricked with a pin. The chart shows that flow was promptly resumed. Thereafter for the next 10 minutes flow took place as it had before the stimulus was given. From the 53rd to the 58th minutes no inflow occurred. The

tip of the tail was again picked up during the 58th minute and pricked with a needle. Inflow made its prompt appearance again, this time after a long period in which no flow had taken place. In the following 10 minutes only two periods of inflow were seen. At the 67th minute the tail was pricked again but no response appeared.

Text-fig. 2 shows the findings in another experiment in which the intermittent inflow was observed for 16 minutes, after which a hypodermic needle was inserted into the tail vein and allowed to remain there. A brief inflow of fluid occurred at once when the tail was first pricked but thereafter the flow of Locke's solution continued into the ear in the usual way. At the 32nd and 34th minutes pricking the tip of the tail with a pin induced inflow at once but failed to do so at the 50th and 58th minutes.

Pain and tactile stimuli, when applied at first, brought about an immediate flow of fluid into the skin of the ear, which lasted but a few seconds. In all the experiments the mere presence of the needle in the tail had no effect, for after the single response to pricking the skin the character of inflow remained as it had been before the needle was inserted and the rate of inflow was not increased.

Blocking the pain and tactile stimuli by novocaine abolished the reaction.

In twelve experiments, 0.1 cc. of 10 per cent novocaine was injected intradermally and subcutaneously at the base of the tails of the mice. In them all, pricking and touching the tip of the tail failed to induce immediate inflow into the skin of the ear. This finding appears in some of the experiments to be described below, for example, that yielding Text-fig. 4, and will not be dwelt upon further.

The observation that pain and tactile stimuli applied to the tail were followed by single brief periods of inflow of fluid rendered it necessary to ascertain whether the needle placed in the skin of the ear might not itself afford cutaneous stimuli giving rise to the intermittent intake observed there. Accordingly, experiments were done in which pain and tactile impulses from the ear were blocked with novocaine.

In fourteen experiments, 2 per cent novocaine was dissolved in Locke's solution and employed in the usual way in the injecting device. 10 minutes before the injecting needle was inserted into the skin, the puncture wound in the ear and the ear itself were painted with the same solution. As Text-fig. 3 shows, the fluid entered the tissues in the usual manner although local anesthesia must have been induced. In these experiments a mild edema, visible under the binocular microscope, appeared in 20 to 65 minutes. In some of them, just before the edema appeared, the periods of inflow took place at longer intervals than usual and each endured for several minutes. This phenomenon is perceptible in the latter portion of Text-fig. 3. After the edema appeared intermittent backflow occurred (not shown in the figure).

It follows from these findings that the intermittent inflow was not produced by sensory stimuli arising from the presence of the motionless injecting needle in the ear.

Are the Methods Employed Sensitive Enough to Detect the Absorption of Interstitial Fluid?

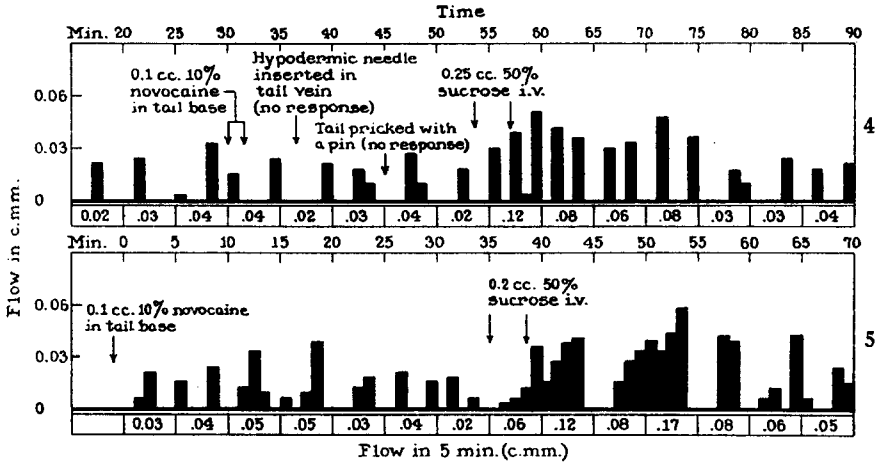
Having determined that pain and touch stimuli cannot account for the intermittency of the entrance of Locke's solution, when brought into contact with the tissues of the skin, as further that the slight effect of the stimuli can be eliminated by novocaine, we took up once again experiments planned to show whether or not the methods employed were sensitive enough to demonstrate changes in the absorption of interstitial fluid into the blood and lymph. It is well known that an increase in the osmotic pressure of blood results in the abstraction of fluid from the tissues. The fact was used to test the sensitivity of the injecting device.

Locke's solution at atmospheric pressure and at body temperature was brought into contact with the dermal tissues of the ears of 24 mice of 27 to 30 gm. body weight. After determining the rate and the character of the intermittent entrance of fluid, 0.2 to 0.25 cc. of 50 per cent sucrose solution was injected into a tail vein. Each injection, representing approximately 3 to 4 gm. of sucrose per kilo of body weight, was given in about 3 to 4 minutes. As is well known, hypertonic sucrose injected intravenously escapes scarcely at all into the tissues (3), and as a consequence by raising the osmotic pressure of the blood abstracts fluid from the tissues. Ellis and Faulkner (4) have shown that in man approximately one-seventh of the dose employed here increased the plasma volume 3 to 17 per cent, on the average 13 per cent, within a minute, the average increase being 9 per cent at the end of half an hour. In every instance in our experiments the blood vessels of the ears were watched under the binocular microscope during the injection. They showed no constriction but the capillary circulation seemed more complete than before.

The findings in a typical experiment appear in Text-fig. 4. It will be seen that prior to the injection of sucrose Locke's solution entered intermittently for 30 minutes into the ear of a 27 gm. mouse, the periods of inflow occurring quite regularly about 3 minutes apart. The first 15 minutes of the experiment are omitted from the figure. At the beginning of the 31st minute, about 1 minute after a period of inflow had occurred and 2 minutes before another was to be expected, the tail was picked up. Immediately fluid entered the ear for a few seconds, the total flow which occurred being shown on the chart in the flow for the 31st minute. During this period 0.1 cc. of 10 per cent novocaine solution was injected subcutaneously at the base of the tail. No further flow occurred within the usual interval of 3 minutes. During the 37th minute a hypodermic needle was inserted into the vein at the tip of the tail without expelling any fluid. There was no immediate entrance of fluid such as occurred in the experiments yielding Text-figs. 1 and 2, in which sensory impulses from the tail were not blocked with novocaine. During the 45th minute the tail was repeatedly pricked with a pin without any apparent change in the flow to the ear. During the 54th minute, after a period of inflow had occurred, an injection of 50 per cent sucrose solution, at body temperature, was begun into a tail vein and 0.25 cc. given during a period of 4 minutes. The injection produced a great increase in the flow of fluid into the ear, as shown in the figure. For 20 minutes following the injection the inflow was either two or three times as great

as before. Nevertheless the flow preserved its intermittent character. Later it returned to the normal rate, as shown in the figure.

In the experiment charted in Text-fig. 5, novocaine was injected into the base of the mouse's tail before measurements of flow to the ear were made. For the first 35 minutes of the experiment the Locke's solution entered into the skin of the ear in the characteristic manner and in the usual amount, the average flow during each 5 minute period



Text-figs. 4 to 8. The absorption of interstitial fluid as influenced by conditions described in the text.

TEXT-FIG. 4. An increase in the osmotic pressure of the blood increases the entrance of fluid into the skin. Hypertonic sucrose solution was injected into a tail vein during the 54th to 58th minutes, inclusive, with result that the flow of Locke's solution into the ear increased. It remained intermittent in character, however. In this experiment novocaine was injected at the base of the tail to block pain and tactile stimuli incidental to the intravenous injection.

TEXT-FIG. 5. The intermittent character of the inflow of Locke's solution is disturbed by an increase in the osmotic pressure of the blood. 50 per cent sucrose solution injected intravenously between the 35th and the 39th minutes increased the take-up of Locke's solution by the skin. Instead of short periods of flow, such as occurred before the injection, there were now two long periods of about 8 minutes each. Thereafter the short periods were resumed.

being about 0.04 c.mm. At the 35th minute the tail was picked up and 0.2 cc. of 50 per cent sucrose solution injected into a vein. The injection required $3\frac{1}{2}$ minutes, as shown in the figure. No immediate entrance of fluid followed the pricking of the tail by the needle, doubtless because of the previous injection of novocaine. Nevertheless, beginning in the 2nd minute of the injection and for 8 minutes thereafter an irregular but continuous entrance of fluid occurred. This was followed 3 minutes later by another period of continuous inflow, which also endured for 8 minutes. During these periods the entrance of fluid into the skin was two to four times as great as it had been previously during similar periods of time. From the 55th to the 65th minutes the flow of fluid

again became intermittent but remained greater than ordinary. For the next half hour flow took place much as it had before the sucrose injection. This is not shown in the chart.

Apparently the increased osmotic pressure of the blood (3,4) had drawn more fluid into the ear from the injecting device. In about half the experiments the flow preserved its intermittent character, as illustrated in Text-fig. 4. In about half of the remainder the intermittent periods of fluid entrance simply became longer, while in the other half a sudden, disorderly, and continuous entrance appeared and lasted for 7 to 15 minutes, as shown in Text-fig. 5.

Of the 24 experiments in which 0.2 cc. to 0.3 cc. of sucrose solution were injected intravenously, an increased entrance of fluid occurred into the ear in 19; in the remaining 5 there was no change in the character or rate of inflow. In 11 other experiments twice as much sucrose was given in periods varying from 5 to 10 minutes. These experiments yielded irregular findings. In 6 the flow increased, in 2 it did not change, and in 3 a slight backflow appeared for 2 or 3 minutes, followed by inflow. Undoubtedly the injection of so large an amount of fluid (0.4 to 0.6 cc. into mice of 27 to 30 gm.) disturbs the circulation markedly.

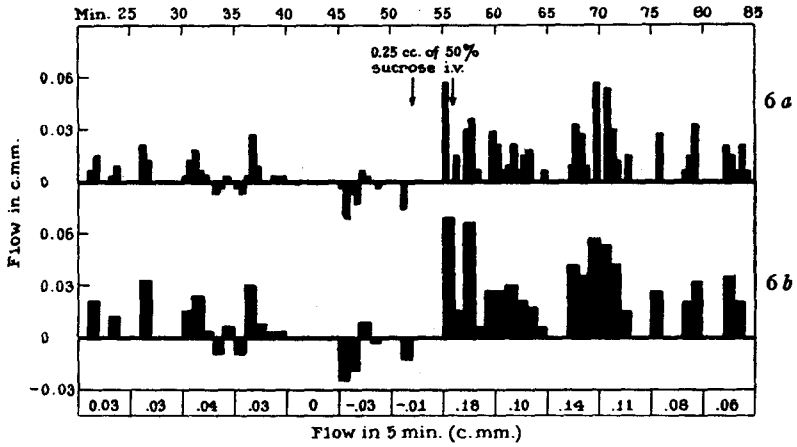
In some additional experiments like those charted in Text-figs. 4 and 5 hypertonic solutions (8 to 10 per cent) of NaCl or 20 to 30 per cent glucose were injected in amounts of 0.2 to 0.3 cc. The findings were irregular; at times increased inflow resulted and at times there was no change. We attribute the irregularity of the results to the fact that sodium chloride and glucose are far more diffusible than sucrose which does not escape readily from the blood stream. The redistribution of tissue and blood fluids following the injection of the former may have been too rapid to register changes in the flow in the apparatus.

The findings described show that the method enables the abnormal absorption of small quantities of interstitial fluid from the skin to be detected, as in these instances in which the blood was rendered hypertonic by experimental means. As already mentioned, the circulation in the ear seemed more complete than before and the vessels dilated; nevertheless there was an increased entrance of fluid into the skin. The findings enable one to suppose that the entrance of Locke's solution into the skin under the ordinary circumstances of experimentation is the result of an intermittent absorption of fluid by the blood, although a series of tissue changes admitting fluid into the tissues irregularly cannot be absolutely ruled out.

Further experiments support this explanation of the findings. We have already mentioned in this and in the preceding paper (1) that in about one-fourth of our experiments the insertion of the injecting needle into the ear of the mouse led, after 30 to 90 minutes, to the development of edema of the skin. In all experiments in which the ears became edematous, the intermittent entrance of fluid at atmospheric pressure ceased and sooner or later backflow into the injecting device occurred. The backflow was always intermittent in character like the inflow. The phenomenon provided an

excellent opportunity to test whether or not intravenous injections of sucrose in these instances, which should lead to resorption of some of the edema fluid by the blood, would result in a renewed inflow of fluid from the injecting apparatus.

Text-fig. 6 *a* and *b* shows the findings in an experiment of this kind, as recorded at 30 second and 1 minute intervals, respectively. As usual novocaine solution was injected subcutaneously at the base of the tail, after which Locke's solution at atmospheric pressure was brought into contract with the dermal tissues of the ear. For 30 minutes



TEXT-FIG. 6 *a* and *b*. An intravenous injection of hypertonic sucrose solution reverses the flow of fluid as the ear becomes edematous. As explained in the text, Locke's solution, which had been flowing into the skin at atmospheric pressure in an intermittent manner, began to move backwards intermittently into the needle as edema of the ear developed. An intravenous injection of 50 per cent sucrose solution caused fluid again to enter the skin. The findings are shown in Text-fig. 6*a* as read at ½ minute intervals, and in Text-fig. 6*b* as read at 1 minute intervals.

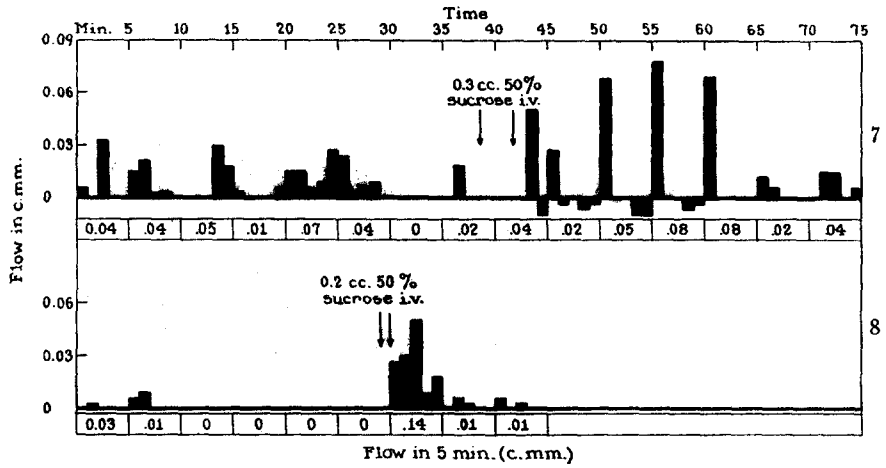
it entered the skin in the usual manner. The findings from the 20th to the 85th minutes are shown in the chart. Between the 30th and 40th minutes the superficial skin near the needle became obviously edematous.

In these experiments the presence of edema could be judged only by observation through the microscope, as it was inexpedient to touch the ear to elicit pitting or pressure. However, many observations of the development of edema of the skin of the mouse ear in scores of previous experiments (2,5–8) in which pitting on pressure could be utilized have taught us to recognize the appearance of its earliest stages.

From the 33rd to the 40th minutes, alternate backflow and inflow of fluid occurred through the needle. From the 40th to the 46th minutes no entrance of fluid took place, and then, in the next 7 minutes, two periods of backflow occurred, as the chart shows. During the 53rd minute an intravenous injection of sucrose was started and 0.25 cc. given in 4 minutes. An irregularly intermittent flow of fluid began before the

injection was completed and, as the figures on the chart show, continued for 20 minutes. Thereafter the entrance of fluid into the skin continued at a slightly faster rate than at the beginning of the experiment, before the injection of sucrose had been given and before the ear had become edematous.

Text-fig. 7 gives the findings in another experiment in which edema appeared in the ear. Instead of Locke's solution, a 2 per cent novocaine-Locke's solution was used in the injecting device, and novocaine was also injected at the base of the tail. For 30 minutes fluid moved into the ear intermittently. Edema began to appear at this time and the flow of fluid practically ceased. Between the 39th and 42nd minutes,



TEXT-FIG. 7. An increase in the osmotic pressure of the blood by intravenous injection of hypertonic sucrose solution increases the intermittent flow of a 2 per cent novocaine-Locke's solution into the skin of the ear, that is to say, under such conditions that pain and touch impulses from the injected ear had been blocked as well as from the tail.

TEXT-FIG. 8. An intravenous injection of hypertonic sucrose solution induces rapid entrance of fluid into the skin. In this instance there had been almost no spontaneous take-up at atmospheric pressure prior to the injection.

before backflow of edema fluid had begun, 0.3 cc. of a 50 per cent solution of sucrose was slowly injected intravenously. For 20 minutes thereafter brief periods of great fluid entrance alternated with periods of slight backflow into the apparatus. As the chart shows, following a 20 minute period of increased flow the entrance of fluid into the ear resumed normal proportions although the ear was still edematous.

It has already been mentioned (1) that in a few of our experiments Locke's solution in contact with the dermal tissues of the ear failed to enter the skin, although there was no edema and no obstruction could be found in the injecting needle. In two such instances intravenous injections of 0.2 cc. of 50 per cent sucrose were given and each time fluid was taken up by the skin. The data from an experiment of this sort are charted in Text-fig. 8. After nearly half an hour, in which almost no fluid entered the skin, an intravenous injection of sucrose produced a large take-up for 15 minutes. Thereafter the flow ceased.

In these experiments the increase in osmotic pressure of the blood presumably drew the edema fluid into the circulation and induced a flow in the injection apparatus. Clearly the method was sensitive enough to detect the entrance of very small amounts of interstitial fluid into the blood.

The Effect of Hyperemia

To test whether or not the intermittent flow taking place into normal skin and into that of the injected animals was caused by periodic absorption into the blood of the fluid rendered available to the tissues by our apparatus, we induced moderate and extreme dilatation of the blood vessels of the ears by a variety of methods. Fluid, at atmospheric pressure, in contact with a tissue which is becoming hyperemic should be pressed upon by the dilating vessels and forced back into the pipette if the intermittent entrance seen under normal circumstances is due to changes in the tissues such that fluid is admitted irregularly. As the following tests show, the development of hyperemia in the ear was accompanied by greater inflow, even when rapid dilatation of the vessels occurred.

The character and rate of the intake of fluid by the skin of one ear of each of 20 mice was observed and measured for about 30 minutes. In half the animals, mild hyperemia was now induced in the ears by shining the light of a Leitz carbon arc lamp upon the back of the animal from a distance of 20 cm., while shielding the head and ears. Warming the body of a mouse or rabbit in this way leads to reflex hyperemia of the ears. The hyperemia was maintained by using the light for periods of 10 minutes followed by variable periods of eclipse. In the other 10 animals, severe contralateral reflex hyperemia was induced in one ear under test by painting the other with xylol.

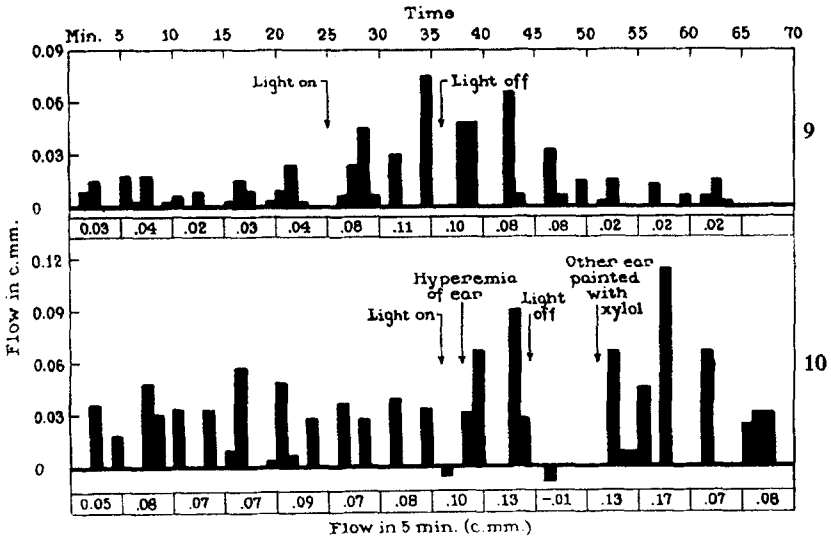
In both types of experiment the flow of Locke's solution into the skin increased despite the fact that both the larger and smaller blood vessels became dilated. In most of these experiments edema of the ear made its appearance sooner or later. Only after the edema had become apparent did the inflow become less than normal or cease. This subject will be discussed more fully below.

In some of the experiments, when the onset of hyperemia was very abrupt, there was a momentary cessation of flow, or more rarely a slight and momentary backflow (see Text-fig. 10) as the vessels suddenly widened, but almost at once, even while they continued to widen, as noted under the binocular microscope, the inflow of fluid increased.

Typical findings from two experiments appear in Text-figs. 9 and 10. In the first experiment the entrance of fluid was watched for 25 minutes, after which, as already described, the animal's body was heated by the arc light for 11 minutes. By the 2nd and 3rd minutes dilatation of the blood vessels had become obvious under the binocular

microscope. As the chart shows, intermittent inflow increased during this period. At the end of the 36th minute the light was turned off. The hyperemia continued to about the 47th minute and so too did the increased intermittent intake of fluid by the skin. When the hyperemia subsided the intake of fluid decreased.

In another experiment sudden severe hyperemia accompanied by extreme dilatation of blood vessels led to increased intermittent inflow of fluid despite the fact that the rate of flow was already high (Text-fig. 10). At the beginning of the 37th minute the back of the animal was heated and within 2 minutes strong hyperemia appeared in the ear. Note the momentary backflow during the 37th minute. The light was turned off during the 45th minute, when edema of the skin first became apparent. Backflow



TEXT-FIGS. 9 and 10. At atmospheric pressure the spontaneous and intermittent flow of absorbable fluid to the skin is increased by hyperemia. In spite of dilatation of the vessels more fluid enters the tissue. See text.

occurred again during the 47th minute. Xylol applied to the animal's other ear at the 52nd minute intensified the hyperemia in the experimental ear and increased the intake of fluid by the skin.

In all of 20 experiments except 4, hyperemia led to increased inflow. In these 4 the rate of entrance of fluid did not alter.

As hyperemia appeared, Locke's solution entered the tissues more rapidly in spite of the increase in the caliber of the vessels. To be sure, as already mentioned, a momentary backflow took place in many of the experiments just as the vessels dilated. No doubt in these instances some of the fluid which had been introduced into the tissues was squeezed backward into the pipette, but there followed immediately an increased intermittent inflow. It seems probable that the intermittency of the latter is due to periodic

absorption and is not referable to intermittent squeezing of the tissue spaces by dilating or contracting blood vessels or muscles.

In most of the 20 experiments in which hyperemia was induced in the ear edema followed its appearance, usually within 5 to 15 minutes. When edema appeared the flow of fluid, at atmospheric pressure, from the injecting device became slower, the periods of no intake lengthened, flow ceased, and finally backflow into the pipette occurred. The backflow was intermittent in character like the inflow.

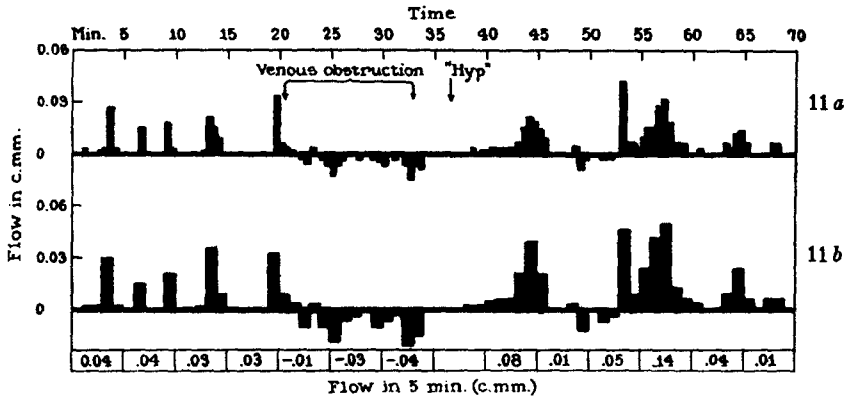
In a few experiments edema did not occur at all or developed three-quarters of an hour or longer after the appearance of hyperemia. It is from such instances that the data presented in Text-figs. 9 and 10 have been taken, because in these the return to a normal intake of fluid by the skin took place after the hyperemia subsided.

The effects of venous obstruction and of subsequent reactive hyperemia were next observed. It is well known that the release of temporary venous obstruction to a part of the body is followed by intense reactive hyperemia (9, 10). Earlier work from this laboratory has shown that lymph flow is greatly increased under these conditions (11). What will happen to an absorbable fluid introduced interstitially into a tissue which is then subjected to venous obstruction? Will the fluid flow backwards into the injecting device? What will happen following release of the obstruction, during the period of reactive hyperemia?

The mouse ear did not lend itself well to experiments with this end in view. The organ is so small that once adjusted, with the injecting needle placed in the skin, further manipulation of the ear, as for example the clamping off of veins, is inexpedient. Hence rabbit ears were employed instead. 6 rabbits, of 2,000 gm. each, were anesthetized with nembutal by intraperitoneal injection of 1.0 cc. of a 5 per cent solution, followed by smaller additional doses as necessary. The Locke's solution was brought into contact with the connective tissue of the animals' ears through a needle introduced into a tunnel, as in the experiments on mice.

Text-fig. 11*a* and *b* gives the results of a typical experiment, recorded in ½ minute and 1 minute intervals, respectively. After 20 minutes of flow, which had the usual intermittent character, the veins at the base of the ear were clamped for 13 minutes, as shown by the arrows in the figure. Backflow into the apparatus almost at once took place and continued as long as the obstruction lasted and for 1 minute after its release. The backflow was irregular but intermittent, as the chart shows. On release of the obstruction an intense reactive hyperemia occurred, at the point indicated by the arrow marked *Hyp* in the text-figure. During this period of great vascular dilatation there was an increased flow of fluid into the skin, which for 8 minutes was continuous. It was followed after a 2 minute period of no flow by two periods of intermittent backflow, which in turn were succeeded by 8 minutes of continuous increased inflow. Thereafter the flow assumed its initial character and quantity.

Similar findings were obtained in the other experiments. During the periods of venous obstruction fluid passed from the tissues into the reservoir, but during the reactive hyperemia it flowed into the tissues at an increased rate despite the dilatation of the vessels. Both the inflow and the backflow were intermittent, from which it follows that the intake or output of fluid in localized regions of skin is not a continuous process.



TEXT-FIG. 11 *a* and *b*. Venous obstruction produces intermittent backflow from the tissues to the reservoir. Reactive hyperemia following release of the obstruction reverses the flow so that much more fluid enters the skin in spite of dilatation of the vessels. The findings are shown in Text-fig. 11*a* as read at ½ minute intervals and in Text-fig. 11*b* as read at 1 minute intervals.

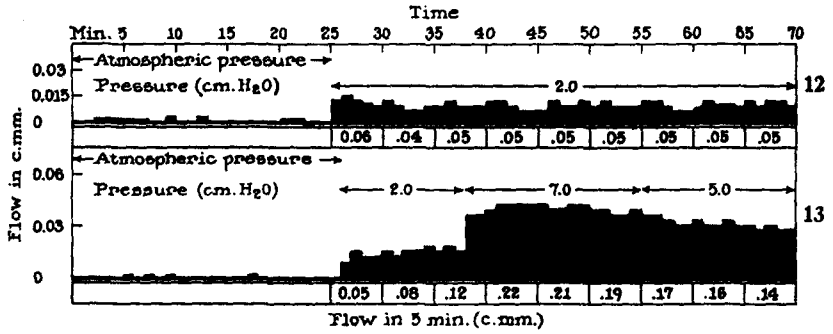
The Influence of the Circulation

If the intermittent movement of fluid into skin is caused by periodic absorption into the blood, cessation of the circulation should eliminate its take-up. The following experiments were done to see whether this was the case.

Locke's or Tyrode's solution was brought into contact in the usual manner with the dermal tissues of the ears of 25 mice 30 minutes or 1 hour after the animals had been killed with ether. In the ears of 20 of these and in the skin of the legs of the remaining 5 there was no inflow of Locke's or Tyrode's solution at atmospheric pressure. As shown in Text-figs. 12 and 13, only dubious movement of the meniscus occurred. Later, in each experiment, a small amount of pressure was put upon the introduced fluid. The result was that fluid entered the skin at rates of flow equal to or greater than that occurring spontaneously into normal skin at atmospheric pressure. But it entered continuously with only slight irregularities in the rate of flow, and there was no real sign of intermittency. We have already shown in

the preceding paper (1) that Locke's solution under pressure moves into living skin in an intermittent manner.

Text-figs. 12 and 13 give typical findings in two of these experiments. The animals were killed with ether 30 minutes and 1 hour, respectively, before beginning the experiment. In each trial, fluid at atmospheric pressure in contact with the tissues for half an hour showed practically no movement. At the times indicated in the charts, pressures of 2.0 to 7.0 cm. of water were brought upon the fluid by a method already described (1). In each instance inflow occurred, but as the charts show it was continuous not intermittent. The irregularities occurring are like those which were noted in control experiments described in a preceding paper and not like those appearing in the tests with living animals, which are also described there.



TEXT-FIGS. 12 and 13. The skin of animals recently killed fails to take up Locke's solution. When forced into the tissue, the fluid moves through it in a continuous manner.

From this it appears that an absorbable fluid under slight pressure enters continuously into the tissues of an animal killed a few minutes before. By contrast (1), it enters intermittently into tissues in which the blood circulates.

Some experiments were attempted in which the entrance of fluid into the skin was first observed for some time in the living mouse, after which the animal was killed by intravenous injections of KCN while the observations were continued. In some of these instances there was no entrance of fluid after death. In others a flow occurred which was very slight in some instances and about like that into living skin in others. These contradictory findings, occurring immediately or a few minutes after cessation of circulation and death, may be attributed to the changes occurring in the ear, agonal spasm of the blood vessels, contractions of the ear muscles, the onset of rigor, or sudden cooling of the ear. To avoid these complications, the experiments already detailed were begun at least one-half hour after death of the animal. Under these conditions the findings were constant.

The Movement of Relatively Unabsorbable Fluids through Living Tissues

To test further whether or not the intermittent flow of Locke's solution into living skin depends upon a periodic absorption of the fluid, experiments were made with a variety of test fluids other than Locke's or Tyrode's solution yet differing from the latter but little if at all in viscosity.

To obtain test fluids which will retain their bulk within the tissues during the experimental periods, we made use of an earlier observation from this laboratory. It had been found (7,5,2) that aqueous solutions of a vital dye, pontamine sky blue, isotonic with blood and sufficiently colored to be seen under the microscope, elicit edema when allowed to come in contact with the tissues.

For the purposes of the present work an aqueous 21.6 per cent solution of this dye, which is isotonic with blood (12–14), was prepared as described elsewhere (2) and added in varying proportions to Locke's solution to yield 5.4 and 10.8 per cent solutions, still isotonic with blood. In 10 experiments, when brought into contact with the dermal tissues of living skin in the usual manner at atmospheric pressure, these solutions showed no movement in the pipette of the injecting device. After varying periods, 5 to 20 minutes, the tissues became edematous close to the point of the needle and intermittent backflow began.

In 6 experiments pressures of 2.0 to 2.5 cm. of water were put upon the test fluids at once, as soon as the needle was introduced into the tissues. As result, for 5 or 10 minutes the dye-Locke's solution was gently forced into the tissues at a rate of 0.03 to 0.05 c.mm. per 5 minutes, the rate at which plain Locke's solution ordinarily enters at atmospheric pressure. The pressure was then taken off the fluid in the needle, in the manner described in the preceding paper, by allowing the butt end of the pipette to communicate with the room air. In all the experiments backflow began at once, and in 3 of the 6 experiments more fluid returned into the pipette, within ½ to ¾ of an hour, than had previously been forced into the tissues.

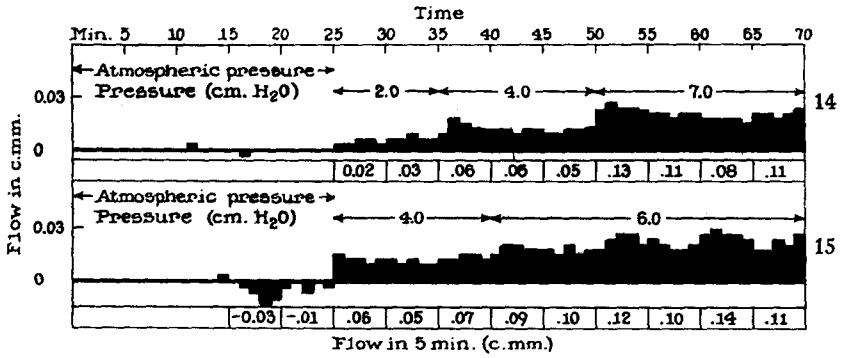
In 6 further experiments, the 5.4 per cent pontamine sky blue-Locke's solution mixture was brought into contact with the tissues in the usual manner at atmospheric pressure for 20 to 25 minutes. In 6 other experiments the 10.8 per cent dye-Locke's mixture was similarly employed. In none did any inflow take place, and in about half the experiments intermittent backflow appeared. In every one of them sufficient pressure was eventually put upon the fluid to force it into the tissues at a rate equal to or slightly greater than the rate of absorption of plain Locke's solution. Entrance was continuous. A chart of a typical experiment with each of the solutions is shown in Text-figs. 14 and 15, respectively. The arrows indicate the points at which various pressures were applied.

As the text-figures show, none of the test fluid moved from the pipette to the tissues at atmospheric pressure, while in some instances there was backflow as edema developed (Text-fig. 15). Afterwards when pressure was exerted the fluids, in both experiments, moved through the tissues in an almost steady stream. There was no intermittency of the flow. Such irregularities of rate as were noted resembled those observed in the control experiments of the preceding paper (1).

In many but not all of these experiments the edema became intense. As it did so an increasing pressure was required to force fluid into the tissues.

In 6 experiments not yet dealt with the 10.4 per cent dye-Locke's solution was run into the tissues under pressure as soon as the injecting needle had been placed in the skin. In these instances, in which edema had not yet occurred, the inflow was more regular than that shown in Text-figs. 14 and 15.

Tests like these were repeated, using the ears of mice killed with ether 30 minutes to 1 hour before. When gently forced into the dermal tissues of the dead animals the movement of the dye-Locke's solution was similar to that shown in Text-figs. 12 and 13. The movement was not intermittent. No charts need to be given.



TEXT-FIGS. 14 and 15. Fluids producing edema are not taken up by the living skin. Forced into it, under pressure they enter continuously. See text.

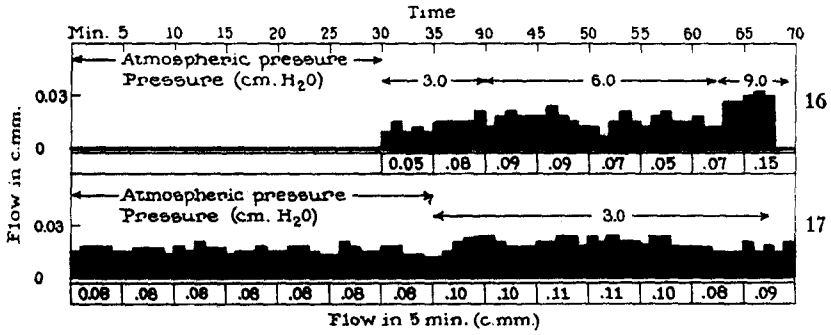
These experiments demonstrate that both the edema-producing dye-Locke's solution and the absorbable, plain Locke's solution move in a similar manner through the skin of animals that have just been killed but behave differently when circulation is going on. That is to say, an edema-producing fluid moves through the ears of living mice in the same way that an absorbable fluid of similar viscosity moves through the ears of killed mice in which there is no circulation. There is obviously no periodic relaxation of the tissues, living or dead, which allows an edema-producing fluid to enter the tissues intermittently.

The Movement of Serum through Tissues.—Part of the movement of absorbable fluid into a tissue, at atmospheric pressure or under positive pressure, may conceivably occur by seepage, like the movement of water into blotting paper. Such inflow would be continuous and would tend to fall off unless the circumstances altered. The intermittent inflow cannot be interpreted in this way but is referable to intermittent absorption. As is well known, serum is absorbed slowly from the tissues of the skin by

reason of its protein content. When it is forced slowly and gently into the skin it enters the tissues continuously. There is no intermittency of inflow like that observed when readily absorbable fluids are forced into the tissue, as described in the preceding paper (1).

A number of mice were bled to obtain fresh serum, which was used to fill the injecting needle and pipette for experiments like those done with the dye-Locke's solutions.

Text-figs. 16 and 17 show the typical findings in 2 of 22 experiments. The behavior of serum was different from that of Locke's solution. In 7 of the experiments there was a slight but steady movement of the serum into the ears at atmospheric pressure. The greatest movement observed is shown in Text-fig. 17. In this instance it amounted to



TEXT-FIG. 16. Homologous serum, introduced interstitially into the skin of some living mice at atmospheric pressure, fails to enter the tissues. Under pressure it moves through the tissues in a continuous manner.

TEXT-FIG. 17. Homologous serum at atmospheric pressure enters spontaneously into the skin of other living mice in a continuous manner. Under pressure its interstitial movement is also continuous.

0.08 c.mm. per 5 minutes. But the flow was not intermittent like that of Locke's solution; only minor irregularities were visible, similar to those in the control experiments (1). In the remaining 15 trials there was no flow, or almost none, at atmospheric pressure, as illustrated in Text-fig. 16. In all these latter instances, enough pressure was put upon the serum, for periods of 30 minutes to 1 hour, to force it through the tissues at the rate of 0.05 to 0.12 c.mm. per 5 minutes, that is to say, at a rate like that at which Locke's solution moved through the tissue when subjected to pressures of 2 to 2.5 cm. of water. In order to attain this rate with serum, pressures of 3.0 to 7.0 cm. of water were usually required. In every experiment the entrance of fluid was continuous.

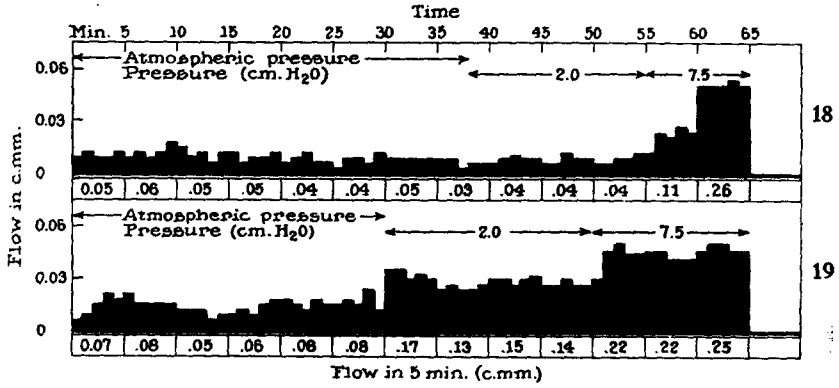
The Interstitial Movement of Serum in the Ears of Dead Mice

Experiments like those just described were repeated using mice killed ½ to 3 hours previously. At atmospheric pressure mouse serum was found to enter the skin of all the dead mice, as it had in about one-third of the experiments on living mice. It did so in a continuous and regular manner,

and when forced into the tissues by gentle pressures (2.0 to 7.5 cm. of water), it again moved without intermission. Indeed, the movement in the ears of dead mice was like that of serum in the ears of the living.

Text-figs. 18 and 19 show typical findings in 2 of 16 experiments. The results need no amplification.

Experiments with Non-Absorbable Fluids.—The findings so far described show that the intermittent flow of absorbable fluid to and from the pipette to the tissues at atmospheric pressure is due to a periodic absorption by the vessels, superimposed doubtless upon passage through the tissues. To test the point further, a final set of experiments was done using a non-absorbable test fluid, purified sperm oil,[1] chosen because of its low viscosity and hence



TEXT-FIGS. 18 and 19. The interstitial movement of homologous serum through the skin of recently killed mice. At atmospheric pressure spontaneous continuous movement into the skin took place. Under pressure the movement was similar but greater.

comparable in that respect to the test fluids already used. The experiments were like those described above save that the oil filled the needle and the pipette of the injecting apparatus. In form the oil meniscus appeared like that of the Locke's solution. As no Locke's solution was used, it was unnecessary to form the paraffin film upon the interior surfaces of the glass. Control experiments, like those described in the preceding paper (1), showed that a pressure of 0.5 cm. of water moved the oil through the pipette and needle of the injecting apparatus in a continuous manner.

After the oil was brought into contact with the tissues at atmospheric pressure, there was no movement whatever of the meniscus in the pipette to indicate any entrance of it or passage backwards such as might have occurred if the oil had induced edema. Further, when hyperemia of the

[1] Merck & Company.

ear was induced by the means already described in this paper, no movement occurred. But when edema of the ears was produced by heat, the accumulating fluid forced the oil slowly backwards along the pipette, as one would expect. No evidence whatever was obtained of intermittent relaxation of the tissues such as might have allowed the oil to enter.

Finally, the oil was forced into the tissues by a pressure equivalent to a column of water 5.0 to 6.0 cm. in height. The oil moved evenly into the ear, with no evidence of intermittent flow.

DISCUSSION

The sources of error which may arise from the employment of our apparatus have been fully considered in the preceding paper. We have used only the findings from experiments in which the pipette remained clean, the meniscus sharp and evenly shaped, and in which there were no signs of hemorrhage or of injury to the ear tissue. Direct injection into blood capillaries or larger vessels or lymphatics has been avoided, as described (1).

The several possibilities which suggested themselves to account for the intermittent flow of absorbable fluids into the skin have already been discussed. Intermittency of flow occurred only in the animal experiments and was not seen when fluid moved through the apparatus alone. It is plain from the circumstances that isotonic fluid brought into contact with the cutaneous connective tissue is rapidly absorbed at certain moments and not at others.

What relationship does this intermittent absorption bear to normal fluid exchange? The amounts of isotonic fluid involved were so minute as perhaps not to have been much greater than those involved in normal exchange and hence there is reason to suppose that the normal turnover of fluid in the mouse's skin is not continuous but intermittent. But the technique employed indicated only that periods of absorption alternated with periods of no absorption. What was happening in the tissues under the latter circumstances we cannot tell. As bearing upon the possibility that fluid was leaving the blood then, it should be pointed out that fluid capable of free interstitial movement is scanty or absent in the connective tissues of the mouse ear and skin (7, 8), never oozing from a microscopic stab wound in normal skin (2). The movable fluid present in normal connective tissues seems to be held by capillary forces; certainly it does not seep from the tissues to the pipette. We have never obtained backflow into the apparatus under normal conditions but only as edema of the skin appeared. From all this it follows that in our experiments upon tissues in a relatively normal state, during the intervals when there was no movement of fluid in our

apparatus, that is to say, between the periods of intermittent entrance of fluid, filtration and absorption between the blood and tissues may have been exactly balanced; or perhaps during short periods of time the output of fluid from the blood may have been greater than the return into it but there was not sufficient accumulation in the tissues to be registered by our apparatus. Since fluid output must have occurred at some time under normal circumstances, and since passage of fluid from the blood cannot be detected by the apparatus until the skin becomes edematous, it is reasonable to suppose that it took place either continuously or only during the periods in which no absorption occurred from the apparatus.

The experiments in which edema formed in the skin and those in which venous obstruction was effected (Text-figs. 6 and 11) indicate that under these circumstances fluid escape from the blood to the tissues was intermittent. Under the circumstances of edema there was but little evidence of absorption of the fluid brought into contact with the tissues experimentally, that is to say, inflow from the apparatus to the tissues. On the contrary, backflow occurred into the apparatus as also under circumstances of induced venous obstruction. Under these conditions, with free fluid present in the tissues any increase of it by fluid output from the blood, or decrease by absorption, should show itself in the apparatus as backflow or inflow, respectively. But there was hardly ever any inflow; only backflow occurred, and this backflow was always intermittent. Much more data illustrating this phenomenon will be given in a later paper in which the conditions in edematous skin will be considered more fully. During the periods in which there was no backflow or inflow to or from edematous skin, or skin subjected to venous obstruction, one may suppose either that filtration and absorption from the vessels were equal or that they were not occurring. During the periods of backflow either passage of fluid from the blood into the tissue became greater than the absorption or the latter decreased or failed.

Can there be superimposed upon the continuous exchange between the blood and tissues an intermittent preponderance, now of escape of fluid, now of resorption? Is it possible that the passage of fluid between the blood and tissues may, now in this spot, now in that, be entirely in one direction, for short periods of time? There are facts which support this supposition.

Blood flow in some tissues is known to be intermittent. Richards and Schmidt (15) noted an intermittent flow through kidney glomeruli. Krogh (16) has reported that the capillaries supplying local regions of the tongue and skeletal muscles of the frog may have blood coursing through them at

one moment and be completely closed at another. Knisely (17, 18) has shown that the supply of blood to certain regions of the spleen is periodic. Grant (19), observing irregularities in blood flow in the rabbit's ear, has discussed these in relation to the possible functioning of arteriovenous anastomoses and has shown that the latter open and close intermittently. The recent studies of Zweifach (20–25) on the circulation in the mesentery, tongue, skin, and intestinal wall of the frog and mesentery and ear of the mouse have emphasized the existence of two sorts of capillaries, "a-v" capillaries which are direct extensions from the arterioles to the venules but are not to be confused with arteriovenous anastomoses, and "true" capillaries which branch off from the a-v capillaries but are smaller than the latter and far more numerous. In the a-v capillaries, which constitute but a small portion of the capillary bed, the flow of blood (or perfused fluid) is continuous, but in the true capillaries flow is intermittent, depending upon the state of circulation in the a-v capillaries. Zweifach's perfusion studies with dyes and particulate matter (21–25) have shown that at times fluids may escape all along the course of a capillary and at other times may enter all along it.

It is well known, too, that capillary pressure varies from time to time in any one region, depending upon local anatomical and functional differences in the circulation (9). Landis (9) has noted that, in an entire capillary or even a whole network of capillaries, the hydrostatic pressure may vary enormously above or below the colloid osmotic pressure of the blood. Such a change, occurring in a capillary network, as result perhaps of the intermittent opening or closing of an arteriovenous anastomosis or changes of flow in true capillaries, could account for the periodic entrance of isotonic fluid into the skin of our experimental animals and might well determine whether fluid passed outward or inward under normal circumstances.

Our results are not adverse to the Starling hypothesis of fluid exchange, for this, as Peters has pointed out (26), refers to the average of processes occurring in the tissues as a whole. They demonstrate that conditions of fluid exchange are constantly changing here and there in the skin, and they go further in suggesting that the passage of fluid to and from the blood may not be an evenly balanced process, controlled solely by the balance between the hydrostatic and colloid osmotic pressures within the capillaries and tissues, but may undergo periodic fluctuations, inflow perhaps preponderating during certain times and outflow at others.

SUMMARY

Minute amounts of Locke's or Tyrode's solution have been brought into contact with the interstitial connective tissue of the skin of the living mouse,

at atmospheric pressure, in such a manner that the blood or lymphatic vessels are not entered directly. Under such circumstances these absorbable fluids enter the tissue spontaneously. Entrance is strikingly intermittent, not continuous, and so too when very slight pressures are brought to bear on the fluids (1).

Hyperemia of the tissues, with accompanying dilatation of the blood vessels, increases the entrance of fluids at atmospheric pressure but it is still intermittent. By contrast, venous obstruction leads to intermittent backflow into the apparatus, but reflex hyperemia, following release of the obstruction, is attended by an increase of flow into the tissues in spite of the great reactive dilatation of vessels. The inflow is also intermittent.

If the skin is deprived of circulation, fluid does not enter it at all at atmospheric pressure, though it moves in regularly and continuously if slight pressure is put upon it. Edema-forming fluids, described in the text, also enter in a continuous manner when forced into the skin of either living or dead animals. So too do serum and sperm oil.

The findings indicate that the passage of interstitial fluid into the blood vessels may be intermittent under normal circumstances and its escape from them as well. The observed occurrence of intermittent flow in the blood vessels of several tissues (9, 15–25) will go far to account for the intermittent entrance of fluid into the skin.

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