AN INQUIRY INTO THE STRUCTURAL CONDITIONS AFFECTING FLUID TRANSPORT IN THE INTERSTITIAL TISSUE OF THE SKIN

By PHILIP D. McMAST, M.D.

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

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The formation of lymph is known to be greatly influenced by hyperemia, edema, muscular movement, injury, or venous obstruction (1-5), but the accompanying changes in the tissues are little understood. Is there an increased pressure upon the extravascular fluid under these conditions, and does this drive it into the lymphatics? Is there a change in the resistance of the tissues to the interstitial passage of fluid from blood to lymph? Knowledge of these matters is essential for an understanding of the processes of lymph formation and fluid exchange.

It seems reasonable to suppose that a study of the resistance of the tissues to the passage of fluid under various conditions may throw some light upon the problem presented. In the present work we have studied the resistance offered by normal and edematous cutaneous tissues to the interstitial passage of fluid. In a following paper we will discuss certain changes in this resistance which are associated with various physiological and pathological states as furthermore, certain changes in the pressure exerted upon edema fluid in edematous cutaneous tissue.

Previous Work.—In 1932 Meyer and Holland (6, 7) attempted to find whether fluids forced through cutaneous tissues move as if in capillary spaces or whether they pass between the formed elements like water filtering through fine sand. The authors pointed out that the ratio of the rate of flow of fluid from a cannula into the tissues should increase in simple proportion to the pressure put upon it if there are tissue spaces like channels, leading fluid away from the cannula (Poiseuille's law). On the other hand, if flow through the tissues is a seepage, like seepage through fine sand, the ratio of rate of flow to pressure should be a quadratic relationship. Meyer and Holland obtained evidence that the ratio of flow to pressure was a direct proportion and concluded that interstitial fluid flowed as if in capillaries.

These findings will be discussed in detail further on. For the present it will suffice to say that the work here to be presented will show that the introduction of fluids into the tissues in the amounts required for the measurements of Meyer and Holland must have pushed apart the formed elements and created artificial interstitial spaces.
Recently in this laboratory methods have been developed (8, 9) by which fluids in microscopic amounts can be brought into contact with the tissues of living skin at atmospheric pressure, or at various known pressures, in such a way that they enter neither the blood vessels nor the lymphatics directly. Under these conditions Locke's solution, at atmospheric pressure, is absorbed into the tissues intermittently (8, 9) and we have been able to measure the rate of its absorption. The technique has afforded an opportunity, as will be explained below, to determine with accuracy the resistance of the tissues to the interstitial movement of various fluids brought into contact with them. Observations have also been made upon the changes in the interstitial movement of Locke's solution and other fluids when brought into contact with the tissues under various positive pressures. The findings throw some light upon the nature of the interstitial spaces and the magnitude of the pressures operating in the tissues during the formation of lymph.

Methods

The technique by which exceedingly small amounts of test fluids have been brought into contact with the tissues has already been described (8, 9). It will suffice to recall here that a gauge 30 needle, carrying fluid from a horizontally placed 0.2 cc. pipette, was introduced into the connective tissue of the skin in such a manner that the fluid brought into contact with the tissues did not pass into the blood vessels or the lymphatics directly (8). The movement of fluid in the pipette, occasioned by the entrance of the former into the tissues, was observed through a microscope and measured by the aid of micrometer eyepieces. As the amount of fluid entering the skin was exceedingly small, it was 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, the apparatus was submerged in a constant-temperature bath (8). An apparatus was also devised, as already described (8), by which the fluid in the pipette and hence the fluid brought into contact with the tissues could be subjected to various pressures. The detailed description of this device, already given (8), need not be repeated here.

The Effects of Pressure upon the Movement of Locke's Solution through the Tissues of the Skin

In 60 experiments we observed the effects of changes in pressure upon the movement of Locke's solution through the tissues of the skin of living mice. It is to be recalled that Locke's solution is readily absorbed from the tissues (9). The experiments yielded consistent results and need not be detailed individually.

The needle and pipette of the injecting device were filled with Locke's solution at atmospheric pressure and the needle carrying the fluid was introduced into the connective tissue of the skin of the ear or the back of mice anesthetized with nembutal (8, 9). The meniscus of the Locke's solution in the pipette was watched continu-
ously. In all of the experiments it was noted, as in our previous work (9), that the Locke's solution at atmospheric pressure entered the tissues intermittently. When the intermittent entrance of fluid into the skin had been observed for 15 to 20 minutes, that is, long enough to determine its rate and character, the pressure of a column of water 1.0 to 2.5 cm. in height was put upon the fluid in the injecting device, by means already described (8). The subsequent movement into the tissues was observed for 10 to 20 minutes, after which pressure in the pipette was again increased by small amounts for an equal period. Later this procedure was repeated, employing slightly larger or smaller increments of pressure and observing the character and rate of fluid movement all the while until pressures of 20 to 40 cm. of water had been utilized. The findings from two experiments typical of the sixty done are presented in Text-figs. 1 a and 2 a, in which the data are plotted as described in our previous papers (8, 9).

In all the experiments the fluid continued to move into the skin at approximately the same rate as before when pressures of 1.0 to 2.5 cm. of water were applied; and in about half of them the intermittency of flow remained unchanged as well. An instance of this sort is illustrated by Text-fig. 1 a. For the first 20 minutes of the experiment the Locke's solution, at atmospheric pressure, entered the tissues intermittently at quite regular intervals. From the 20th to the 35th minutes, inclusive, as indicated by the numerals between the horizontal arrows near the top of the text-figure, a pressure of 2.5 cm. of water was put upon the fluid in the injection apparatus. Neither the rate nor the character of the flow changed appreciably.

In the other half of the experiments pressures of 1.0 to 2.5 cm. of water produced slight alterations in the intermittency of flow, but the rate of flow did not increase. The periods of inflow lasted a little longer, or recurred at shorter intervals, and the periods of no flow were correspondingly shortened. The data from a typical experiment of this sort appear in Text-fig. 2 a. In certain instances, of which this is one, the changes just mentioned became more obvious when increasing pressure was put upon the Locke's solution. (Vide Text-fig 2 a, from the 30th to the 45th minutes, when a pressure of 3.5 cm. of water was employed in the injection apparatus. It will be seen that the rate of flow into the tissues increased only slightly.)

In practically all of the experiments the flow became continuous when the pressure was raised to approximately 4.5 cm. of water. Text-figs. 1 a and 2 a both show this fact. The intervals of no fluid movement were replaced by periods of steady flow, separated from one another by periods of slightly greater inflow. It was as if a continuous passage through the tissues had been superimposed upon the ordinary intermittent inflow. In about half the experiments, as e.g. in that charted in Text-fig. 1 a, there was no change in the rate of entrance of Locke's solution into the tissues, while in the remainder, as typified in Text-fig. 2 a, there was an insignificant increase. In all of the experiments further increases of pressure up to 7.5 cm. of water produced hardly any further change, the intake of Locke's solution increasing but little and in many instances (none of which is shown in the text-figures) not at all.
The interstitial movement of Locke's solution into the skin of a living mouse at various pressures. The narrow black columns represent the amount of flow of the solution into the skin during each minute of the experiment. A heavy base line has been drawn to indicate that the observations were continuous. The method of plotting the text-figures has been described and discussed in preceding papers (8, 9). In this and all the similar charts, the pressure put upon the introduced fluid is indicated, in centimeters of water, by the numerals placed between the horizontal arrows near the tops of the text-figures.

It will be seen that at atmospheric pressure spontaneous intermittent inflow occurred. Neither the manner nor the rate of inflow was appreciably influenced when pressures of 2.5 and 4.5 cm. of water were put upon the fluid. Only a slight increase in flow was noted when the pressure was 4.5 cm. of water. A pressure of 6.0 caused little further change, and a pressure of 7.5 but little more. At a pressure of 8.8 cm. of water a great and sudden increase of flow occurred, as though the tissues had been forcibly pushed apart.

The data from the same experiment plotted to show the average rate of entrance of Locke's solution into the skin. It will be seen that inflow increased but little over that occurring at atmospheric pressure when pressures ranging from 1.5 to 7.5 cm. of water were employed. Flow was greatly increased by a pressure of 8.8 cm. of water, and proportionately increased at higher pressures.
Very different were the findings in every one of the experiments when higher pressures were used. The changes then occurring are well illustrated by the typical experiments of Text-figs. 1 a and 2 a. In both cases, when the pressure on the introduced fluid was increased from 7.5 to 8.8 and 8.5 cm. of water, respectively, there suddenly occurred a great increase in the rate of flow into the tissues, more than quadrupling the previous flow. The great increases in flow followed upon increases in pressure only 1.0 and 1.3 cm. of water higher, respectively, than the pressure previously employed. Apparently at some pressure between 7.5 and 8.5 to 8.8 cm. of water there was a sudden change in the resistance of the tissues to the flow of fluid through them. When this “breaking point,” as we shall term it, had been reached, the inflow abruptly became so great that the slight differences produced by the original intermittency were no longer significant, although they still seemed to occur. When in these experiments and in others to be described and discussed below, still greater pressures, up to 20 or 40 cm. of water, were put upon the fluid introduced into the tissues, each further increase in pressure yielded proportionately greater inflow. The data on this point have not been included in the charts.

The “breaking point” appeared in every experiment. In a few of them it appeared at pressures as low as 4.5 cm. of water, in a few others not until pressures as high as 12.5 or 14.0 cm. had been brought to bear. In more than 80 per cent of the experiments (49 trials) the “breaking point” appeared at pressures varying from 6.5 to 10.0 cm. of water, and it averaged 8.5 cm. for them all.

The changes in the rate of passage of Locke’s solution into the tissues as affected by the various pressures are strikingly shown when the average rate of inflow per 5 minutes at each pressure is plotted. In Text-figs. 1 b and 2 b this is done for the two typical experiments already considered. A sharp elbow in each curve marks the “breaking point.” Additional data not plotted in Text-figs. 1 a and 2 a have been included, which show that after the “breaking point” had been reached each small increase in pressure put upon the Locke’s solution produced some increase in its flow through the skin. More will be said of this phenomenon below. In Text-fig. 3 we have plotted the findings from four other experiments, selecting instances to typify “breaking points” that were low, average, and high, respectively.

Text-fig. 3, line A, shows the flow occurring at different pressures in an instance in which the “breaking point” was reached between 4.2 and 4.8 cm. of water. In this experiment Locke’s solution at atmospheric pressure entered the tissues at an average rate of 0.12 c.mm. per 5 minutes. At pressures of 2.2, 3.0, and 4.2 cm. of water, flow was only 0.13, 0.14, and 0.14 c.mm. per 5 minutes, respectively. At a pressure of 4.8 cm. of water a sudden increase in flow occurred, which became proportionately greater with increasing pressures.
Text-Fig. 2 a. Data from another experiment of the sort plotted in Text-fig. 1 a. In this instance pressures of 2.5 to 4.5 cm. of water did not importantly influence the rate of flow but produced slight alterations in the intermittency of the flow. The periods of inflow endured a little longer or they took place at shorter intervals than they had at atmospheric pressure.

Text-Fig. 2 b. Data from the same experiment plotted as in Text-fig. 1 b.

Lines B, C, and D show similar data in three other typical instances. In two of them the "breaking points" lay between 7.0 and 9.0 cm., the average level; while in the third it was not reached until a pressure of 12 cm. of water was exerted.

On comparing these four lines a significant point will be noted. In each experiment measurable flow occurred spontaneously at atmospheric pressure.
Upon the application of low pressures, from 2.0 cm. of water upward until the “breaking point” was reached, there occurred very little increase in the rate of flow, sometimes none. After the “breaking point” had been reached, each addition of pressure produced such an increase in flow that there was a linear relationship between the two, as though the latter were taking place through small channels. This interpretation will be discussed more fully below.

Text-Fig. 3. The data from four typical experiments, plotted as in Text-figs. 1b and 2b, to illustrate further the effect of changes in pressure upon the rate of entrance of Locke's solution into the skin. Instances of high, low, and average “breaking point” (see text) have been selected. There was a linear relationship between pressure and flow after the “breaking point” had been exceeded.

The Resistance of the Tissues to the Entrance of a Relatively Unabsorbable Edema-Producing Fluid

In the work just reported, such minute amounts of fluid were introduced into the skin, even under pressure, that one could not be certain how much of the increased flow from the apparatus was due to a greater movement of fluid through the tissues and how much resulted from absorption by the blood vessels. The next experiments bore upon this point. They were carried out with a fluid similar in viscosity to Locke's solution but one which calls forth edema, augmenting the fluid bulk within the tissues.

To accomplish our end we have utilized a finding made previously in this laboratory and reported in an earlier paper (9). It was found that the addition to Locke's
solution of ½ per cent of a vital dye, pontamine sky blue, yielded a mixture which failed to enter the cutaneous connective tissue when brought into contact with it at atmospheric pressure. On the contrary, when the mixture was in contact with the skin it called forth an accumulation of fluid in the tissues and after a few minutes this accumulation began to force its way into the injection apparatus, so that the fluid already in the pipette was moved backwards.

In many other previous experiments (9) the mixture of dye and Locke's solution was brought into contact with the tissues at atmospheric pressure and immediately thereafter forced into the skin at low pressures, that is to say, before fluid had accumulated in the tissues in sufficient quantity to reverse the flow in the injection apparatus or to give rise to an edema visible under the microscope. Under these circumstances the dye-Locke's solution entered the tissues continuously, showing none of the intermittent movement that appears when plain Locke's solution is employed in the same manner. As the phenomenon has been described and the data fully charted in two preceding papers (8, 9), it need not be detailed further.

In the present work, in 40 experiments, the dye-Locke's solution at atmospheric pressure was brought into contact in the usual way with the connective tissue of the skin of the ears or thighs of anesthetized mice. The fluid did not enter except in two instances, which were discarded. In each of the remaining experiments, after it had been ascertained that the dye-Locke's solution failed to enter the tissues during a period of 10 to 15 minutes, the pressure was increased in the injecting device for equal periods of time and by small increments, as in the preceding experiments. When pressures varying from 1.5 to 3.5 cm. of water were applied, the dye-Locke's solution entered the tissues at rates varying between 0.03 and 0.09 c.mm. per 5 minutes, that is to say, at a rate resembling that of the spontaneous entrance of plain Locke's solution at atmospheric pressure. The inflow was not intermittent as in the experiments made with plain Locke's solution, Text-figs. 1 a and 2 a, but on the contrary was continuous and irregular, as in the experiments of our earlier work just mentioned (8, 9).

From these findings it is apparent that there were factors making against the entrance of the dye-Locke's solution and that these were overcome by pressures of 1.5 to 3.5 cm. of water. As in the trials with plain Locke's solution, the rate of inflow was increased but little, sometimes not at all, by pressures ranging from 1.5 to 7.5 or 8.0 cm. of water. But with a greater increase a "breaking point" was reached at which a slight further addition in pressure brought about a great and sudden increase in flow. In rare instances the sudden inflow of fluid took place at a pressure of 5.0 cm. of water, and in occasional trials it did not occur until pressures as high as 11.0 to 12.5 cm. of water were exerted, but on the average it occurred at a pressure of 8.5 cm.

Text-figs. 4 a and 4 b show the results of a typical experiment. It will be seen that the findings differ from those of the previous charts in the ways just
mentioned above. There was no inflow of the dye-Locke's solution mixture at atmospheric pressure. When forced into the tissues at pressures of 1.9,

Text-Fig. 4 a. The passage into the tissue of an edema-forming fluid, dye-Locke's solution. The data, plotted as in preceding figures, show that no fluid entered the tissue at atmospheric pressure, and that it entered continuously at pressures of 1.9 and 3.0 cm. of water. There was little increase of flow when the pressure was increased to 4.5 and 7.5 cm. of water, but finally, a sudden, great inflow at a pressure of 9.0 cm. of water. Evidently the "breaking point" had been exceeded.

Text-Fig. 4 b. The data of the experiment from which Text-fig. 4 a was drawn, plotted to show, as in Text-figs. 1 b, 2 b, and 3, the changes in the average rate of entrance of the dye-Locke's solution at various pressures.

3.0, 4.5, and 7.5 cm. of water, the flow was not intermittent but continuous although irregular, showing that the resistance of the tissues to the entrance of the fluid was overcome. A pressure of 9.0 cm. of water produced a fivefold increase in the rate of the entrance of fluid. The findings at pressures higher than 9.0 cm. of water have been omitted from the figure. In Text-fig. 4 b we
have charted the data from this same experiment to show the average rate of inflow for each 5 minute period at the various pressures employed, up to 15 cm. of water.

Text-fig. 5 shows the data from four typical experiments of the sort plotted in Text-fig. 4 b. In some of the four, as e.g. in this experiment, there was little change in either the rate or the manner of fluid entrance into the skin at pressures below the "breaking point"; in some the rate of flow increased slightly; while in others an increase in pressure resulted in a decrease in the observed flow of fluid into the skin. It is clear from this that the observed

![Text-Fig. 5. Changes in the average rate of entrance of dye-Locke's solution at various pressures. The data are plotted as in the preceding text-figure. Each increase in pressure after the "breaking point" had been exceeded brought about a corresponding increase of interstitial flow, as evidenced by the straightness of the lines.](image)

differences in flow at different pressures below the "breaking point" often fell within the margin of error of the method. In all the experiments, after the "breaking point" had been reached each increase in pressure brought about a corresponding increase in the flow of the dye-Locke's solution through the tissues. As result, the later slant of the curves is approximately straight.

Resistance to the Entrance of Serum

Experiments like those just described were repeated, using homologous serum, a relatively unabsorbable and viscous fluid. It is well known that serum injected interstitially is absorbed slowly.

Fresh, sterile mouse serum obtained from pooled specimens of mouse blood taken with aseptic precautions was brought into contact with the tissues of the skin of the
ears or thighs of 34 anesthetized mice. In about half the number, as in previously reported work (9), the serum, at atmospheric pressure, entered the tissues at the extremely slow rate of 0.01 to 0.02 c.mm. per 5 minutes, about a third the rate of Locke's solution under similar conditions. In the other tests no fluid entered. Only these instances were employed in the present work. After 15 to 20 minutes had elapsed with no entrance of fluid, the pressure within the apparatus was raised by stages, as in the preceding experiments, until at last flow took place at a rate like that of the spontaneous flow of plain Locke's solution into the skin at atmospheric pressure. It was found that pressures of between 1.5 and 4.5 cm. of water sufficed to bring this about. The flow was continuous and slightly irregular like that occurring in the experiments made with dye-Locke's solution as just described.

As in the experiments with the dye-Locke's mixture or plain Locke's solution, further slight increases in pressure produced little increase in flow until a "breaking point" was arrived at. Then there occurred a sudden and greatly increased flow.

The findings of a typical experiment appear in Text-figs. 6a and 6b. They show that the "breaking point," as indicated by the commencement of sudden, rapid inflow into the skin, occurred at approximately the same pressure as in the tests made with Locke's solution or with the dye-Locke's mixture. In the experiments in which serum was employed the increase of inflow after the "breaking point" had been reached was not so great or so abrupt as in the trials employing the other solutions.

The Nature of the "Breaking Point"

Findings in the Skin of Dead Animals.—In the experiments so far described the "breaking point" was reached at the same pressure whatever the nature of the fluid employed. One may infer therefore that it was determined by the mechanics of the situation, by the bulk of the fluid introduced into the tissues whereby some structural change or separation of the formed elements was effectuated. To exclude the possibility that a circulatory change might have been responsible for the sudden entrance of fluid into the tissues, experiments like those described were repeated on animals that had been killed with ether 1 to 5 hours previously.

Plain Locke's solution, dye-Locke's solution, and homologous serum were used, respectively. We have already shown in a preceding paper (9) that Locke's solution and the dye-Locke's mixture at atmospheric pressure fail to enter the skin of killed mice. Homologous serum also has failed to enter in about half of the trials made, and it passed into the skin very slowly in the remainder. When pressure was brought to bear on these fluids, all three entered into the tissues continuously at pressures of 1.5 to 5.0 cm. of water and there was no sign of the intermittency of flow that appears when Locke's solution is brought into contact with living skin at atmospheric pressure or forced into it at low pressures.
Text-figs. 7 and 8 (a and b) show the results in two typical experiments out of 26 made on killed mice. In the experiment illustrated by Text-fig. 7, plain Locke's solution was employed; in that represented by Text-fig. 8, a mixture of Locke's solution with 3% per cent of dye. In both instances the test fluids failed to enter the tissues when brought into contact with the skin at atmospheric pressure. Subjected to pressures of 2.0 to 5.0 cm. of water, the fluids entered the tissues in a continuous manner, and there was almost no sign of the intermittent flow which takes place when Locke's solution is forced by similar pressures into living skin (9). It will be noted that in both experiments a sudden increase in the rate of entrance of the test fluids ("breaking point") appeared when the pressure of the introduced fluid was raised to 7.0 and 9.5 cm. of water, respectively.

The findings in these two experiments were like those obtained with living animals except for the fact that plain Locke's solution did not enter the skin at atmospheric pressure. The other twenty-four experiments of this group yielded similar results. "Breaking points" appeared at the same pressures...
Text-Fig. 7 a

Text-Fig. 7 b

Text-Fig. 8 a

Text-Fig. 8 b

Text-Figs. 7 a, b, and 8 a, b. The rate of entrance of Locke's solution (Text-fig. 7) and of edema-forming dye-Locke's solution (Text-fig. 8) at various pressures into the skin of recently killed mice.
as in the experiments on living animals, that is to say, at a pressure of about 8.5 cm. of water on the average. In rare instances “breaking points” appeared at a pressure of 5.0 cm. of water or failed to appear until the pressure was raised to 12.0 cm. of water. The findings showed clearly that the resistance to the entrance of fluid into the skin of recently killed animals is like that offered by the skin of the living. It follows that the circulation has nothing to do with it. The conclusion seems justified that the “breaking point” is due to the giving way of some structural barrier to inflow.

The Entrance of Fluid into Edematous Skin

Edema of the skin much affects the entrance of fluid.

In 39 experiments edema was induced in the skin of the ear by painting it with xylol. This procedure had been found effective in inducing edema in scores of earlier experiments (10-14), as shown by the appearance of the skin under the microscope and by “pitting on pressure” exerted with a small needle. The edema was pronounced in the present experiments and the appearance of the skin proved a sufficient indicator.

In all of the 39 experiments the dye-Locke’s solution was employed and observations on the entrance of fluid into the tissues were made as already described. In fourteen cases observations were begun 15 to 20 minutes after painting the ear with xylol, that is to say, while the edema was developing (12). In ten of the remaining 25 experiments tests were made 1½ to 6 hours after painting the ear with xylol, in eight instances 20 to 24 hours later, and in seven instances 4 to 11 days later. In them all the test fluid was brought into contact with the connective tissue of the skin in the usual way and the fluid reservoir was then opened to the atmosphere, to find whether the tissue contained freely movable edema fluid under pressure. When that was the case it flowed back into the pipette. The backflow was always intermittent. It occurred in twelve of fourteen instances in which studies were made within an hour after painting with xylol, that is to say, while edema was developing. It also took place in all of the ten instances investigated 1½ and 6 hours after induction of edema, but only in half of the eight studied 20 to 24 hours after the xylol painting, despite the fact that the ears were still swollen. Still later, 4 to 11 days after edema had been induced, backflow occurred into the injection apparatus in six of the seven trials made. In all seven edema of the skin was still visible at this time.

After it had been ascertained that edema fluid was present, the pressure in the injection apparatus was raised by small increments for varying periods of time and the changes in the rate of flow inwards of the dye-Locke’s solution were followed as in the preceding experiments.

In Text-fig. 9 the findings from seven typical experiments are given, to show the changes in the rate of inflow of the dye-Locke’s mixture when introduced into the edematous skin under various pressures. The curves are plotted as in Text-figs. 3 and 5. We have omitted from them the initial backflow into the apparatus which occurred in most of the experiments when the pipette was opened to the atmosphere. It is noteworthy that in most of the instances
there was no well defined elbow in the curve, at best an ill defined one, or often indeed none at all, like that which is indicative of the existence of a "breaking point" when fluid is forced into normal skin (vide Text-figs. 3 and 5 and the other figures plotted in the same way). The instances showing evidence of an elbow were usually those with the least or most recent edema, as will appear further on.

![Graph showing the rate of entrance of dye-Locke's solution at various pressures into edematous skin.](https://example.com/graph.png)

**Text-Fig. 9.** The rate of entrance of dye-Locke's solution at various pressures into edematous skin. The relationship of pressure to flow is plotted as in the preceding text-figures of the same sort. In contrast with what they show, each small increase in pressure in the present instance was attended by a significant increase in the rate of flow. In some instances there was nevertheless a "breaking point" beyond which fluid entered faster.

Lines 1, 2, 3, and 4 represent the findings in four of the fourteen experiments made an hour or less after painting the ears with xylol, that is to say, during the formation of edema. Lines 1 and 2 represent typical findings from instances which showed much free edema fluid, as judged by the amount of backflow into the apparatus during the preliminary test of conditions. Line 3 is a typical curve plotted from the findings in an instance which showed very little edema fluid, and line 4 is taken from one of the two instances of the fourteen studied during the development of edema, in which there was no backflow into the apparatus although the skin was obviously edematous. It is to be noted that lines 1 and 2 do not mount like lines 3 and 4, that is to say, relatively low pressures had a much greater effect on the entrance of fluid in the experiments from which the first pair of lines was drawn. Further, it is of
interest that lines plotted from experiments in which there was little or no
demonstrable edema fluid, lines 3 and 4 respectively, show an elbow, as if the
edema had not wholly done away with a "breaking point." These findings
are typical of the data from experiments that have been omitted from the
figure for the sake of simplicity. In about half of the instances showing much
edema fluid the findings were generally like those plotted in line 1, yielding
no evidence of a "breaking point," whereas in the others a fairly well defined
elbow appeared, as in line 2, in spite of the presence of edema fluid.

The two lowest lines, 5 and 6, in Text-fig. 9 show the findings in two in-
stances typical of the ten experiments in which the tests were begun 1½ to 6
hours after painting the ear with xylol. In every instance backflow occurred
at atmospheric pressure, showing that there was free fluid present in the skin.
Lines 5 and 6 are drawn from experiments made 5 and 4 hours, respectively,
after painting the ear with xylol. In both of these instances there was much
backflow into the apparatus at the beginning of the experiment when the
dye-Locke's solution was brought into contact with the tissues at atmospheric
pressure. The dotted line 7 gives the findings in an experiment made 5 hours
after painting the ear with xylol. In this instance there was only a little
demonstrable edema fluid and the line slants more sharply upward than lines
5 and 6. It is plain that the test fluid introduced into the skin in this experi-
ment at increasing pressures did not pass into the tissues as readily as in the
instances that showed much free edema fluid. Nevertheless there was no evi-
dence of a "breaking point."

The findings from the experiments made 20 to 24 hours or more after painting
the ears with xylol are not shown, for when plotted the data yielded lines
similar to those numbered 5 and 6.

The findings plotted in Text-fig. 9 have been selected as typifying the
changes that occurred, but deviations were frequent. The rate of movement
of fluid introduced at a given pressure was not always greatest when the skin
contained most fluid as evidenced by the grade of edema, nor was the flow
inwards at a given pressure always greater in ears edematous several hours
than in those painted only an hour before. Some of the individual differences
can be explained no doubt by differences in the pressure under which the
edema fluid was held, or by differences in the interstitial pressure, a factor to
be discussed in a later paper.

As already noted, in some instances if the edema fluid in the tissues was
apparently scant, there was suggestive evidence of a partial "breaking point,"
as indicated by a suddenly lessened slant of the plotted lines.

On comparing Text-fig. 9 with Text-figs. 1 b to 8 b, inclusive, it will be seen
that the lines in the latter run almost vertically in the early part of the experi-
ments, showing that pressures between 2.0 and 8.0 cm. of water effected no
significant increase in the movement of fluid into the skin. Not until the
"breaking point" was reached did a significant increase in flow take place. In Text-fig. 9, on the other hand, the slope of the lines shows that each small increase in pressure above that required to initiate flow into the tissues resulted in a significant increase in the rate of flow. This was true, to a greater or less extent, in all of the 39 experiments on edematous ears. The lines are far from vertical in the first portion of Text-fig. 9 and as the pressure was raised many of them became approximately straight. The significance of this difference will be discussed below.

DISCUSSION

The findings throw light upon the manner of movement of interstitial fluid through connective tissues. The resistance of dermal tissue to the entrance of the test fluids at the rate at which Locke's solution is taken up at atmospheric pressure was negligible. But the skin offered a definite obstacle to the entrance of fluid at a faster rate. Regardless of the fluid employed, the rate of flow into the skin did not increase appreciably as pressure was increased, until a "breaking point" was reached. Since no relationship was found between the rate of fluid entrance into the skin and the pressure employed until this point had been attained, the findings yielded nothing to suggest the presence of preformed spaces or channels through which fluid might stream. On the contrary they indicated that there are no such spaces or channels. But after the "breaking point" had been reached each further increase in pressure led to proportionate increases in the rate of flow of the introduced fluid irrespective of the character of this, with result that a linear relationship developed between the increases in pressure and the flow. This was roughly constant for each animal but differed from individual to individual, as the slope of the lines in the text-figures show.

At pressures above the "breaking point" fluid moved through the tissues as though in small spaces or channels. From the fact that the "breaking point" occurred at the same level regardless of the fluid employed, one can infer that it was determined by the mechanics of the situation, by the bulk of the fluid introduced. The possibility that circulatory changes could account for it has been ruled out by the experiments which showed that it occurred at the same pressure in the skin of living animals and in those killed with ether. Obviously it signified a separation of the formed elements, resulting from the pressure of the introduced fluid. Inevitably such a change must always occur when fluids are injected into tissues by hand, as in clinical medicine, for under these circumstances, as will be shown in later work, the pressure of injection is far higher than that required to exceed the "breaking point."

This abrupt change in the characteristics of fluid movement through the interstitial tissue of the skin makes plain a fact suggested by earlier work (13, 14), that interstitial fluid does not exist normally in tissue spaces large enough to permit it to move freely.
When edema was present in the skin the characteristics of interstitial fluid movement changed much. After flow into the skin had been initiated, by exerting pressure, at the rate at which Locke's solution is taken up by normal skin at atmospheric pressure, each increase of pressure, however slight, led to a corresponding increase in the rate of flow. The latter was more rapid than in normal skin at corresponding pressures, showing that the resistance of the edematous skin to the passage of fluid at these pressures was less than normal. The relationship of this change to the formation of lymph in edematous skin will be discussed in a following paper after data have been presented on the pressure of edema fluid under these circumstances.

In more than half the instances of edema studied no “breaking point” appeared as the pressure was raised and throughout the experiment a linear relationship continued to exist between the pressure employed and the rate of flow. It is known that in edematous skin the formed elements of the tissues are separated slightly by the fluid. One might expect that the entrance of more fluid under these circumstances would take place as if through preformed spaces, and our findings show that this is actually the case.

In certain of the experiments upon edematous skin, a fairly well defined “breaking point” did appear. These were usually instances in which the skin had only recently become edematous (Text-fig. 9). One may infer that under such conditions spaces may not have opened up to the same extent as later and that in consequence the tissue may yield further when pressure is brought to bear.

One further point deserves mention. As already stated, Meyer and Holland (6, 7) concluded from their work that fluids move through normal tissues as though through capillary spaces. The reason for their conclusion is now clear. They forced 0.6 per cent NaCl solution into the subcutaneous tissues at pressures much above the “breaking point” demonstrated in the present work. Indeed they injected the fluid into the tissues at the rate of 30.0 to 40.0 c.mm. a minute. Having ascertained the pressure required to yield that rate of flow, they lowered the pressure until the saline solution entered the tissues at the rate of 10.0 c.mm. a minute. Plotting the rate of flow against the pressure employed, they estimated by extrapolation the pressure at which no flow would theoretically occur. In each experiment the tissues must have been forced apart to begin with by pressures above the “breaking point” and thereafter the rate was measured of flow through the artificial tissue spaces that had been created.

**SUMMARY**

With the aim of determining the structural conditions which affect fluid movement in the cutaneous connective tissue of mice, various test fluids were brought into contact with it under conditions such that neither blood vessels
nor lymphatics were directly entered. Locke’s solution, mouse serum, and a mixture of Locke’s solution with a dye which causes edema were all employed. At atmospheric pressure, Locke’s solution entered the tissues intermittently. When subjected to very low pressures it continued to enter the skin intermittently and at approximately the same rate. At pressures above 4.5 cm. of water, however, the flow became continuous but it did not increase in rate significantly until pressures of about 8.5 cm. were employed. There was no relationship between the rate of flow and the pressure employed. At a pressure of about 8.5 cm. the resistance of the tissues seemed to give way abruptly as if the formed elements had been separated. This has been termed the “breaking point.” After it had been reached each further increase of pressure produced a proportionately greater inflow.

Under the conditions of our experiments, the dye-Locke’s solution and also the homologous serum failed to enter the tissues at atmospheric pressure. It was necessary to subject these fluids to pressure to force them into the skin at the same rate at which the Locke’s solution entered it spontaneously. Under these circumstances the dye-Locke’s solution and the serum entered the skin continuously, not intermittently like the plain Locke’s solution. As the pressure was gradually raised, no significant increase of flow into the tissues occurred until a point was reached, on the average 8.5 cm. of water, at which fluid suddenly began to enter very rapidly. This point, the “breaking point” already mentioned, was reached at the same pressure irrespective of the character of the fluid employed, showing that the phenomenon was produced by the fluid bulk. Once it had been attained, further increases in pressure caused proportionately greater inflow of fluid. The circulation had nothing to do with the phenomenon, for it occurred in the skin of dead mice.

The findings indicate that under normal circumstances the movement of fluid in the interstitial tissue does not take place as though in preexisting channels. The experiments confirm previous observations from this laboratory (13, 14) that in normal skin tissue the state of affairs is such that fluid cannot flow freely. However, when fluid is introduced into the skin under pressure spaces are forcibly opened up.

Inflammatory edema in the skin changed the phenomena of fluid entrance into it under pressure. The reason is that there then occurred a separation of the formed elements and the interstitial fluid moved as in preformed channels. Even when very low pressures were employed (3.0 to 7.0 cm. of water), there appeared usually a linear relationship between the pressure and the rate of flow.

BIBLIOGRAPHY