THE EFFECT OF THE PULSE ON THE SPREAD OF SUBSTANCES THROUGH TISSUES

By PHILIP D. McMASTER, M.D., and ROBERT J. PARSONS, M.D.

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

(Received for publication, June 8, 1938)

Pulsation of the blood vessels in the ear of the rabbit increases the formation and the flow of lymph, as the preceding paper (1) has shown. In the present work we report the results of an investigation of the effect of the pulsation of blood vessels upon the interstitial spread of vital dyes through the skin of intact ears of normal rabbits, and through that of amputated ears perfused with defibrinated rabbit's blood.

Methods

To measure the spread of materials through the tissues of the rabbit's ear isotonic solutions of two vital dyes, pontamine sky blue and patent blue V, were employed. The former, a relatively indiffusible dye, was made up in 2 and in 21.6 per cent aqueous solutions isotonic with blood. The patent blue V, a highly diffusible dye, was employed in 11 per cent aqueous solutions, also isotonic with blood. Both dyes have been used in our previous studies upon the physiology of the lymphatics (2–7). The solutions were introduced without pressure into the tissues of the rabbit's ear in the following manner. With a dissecting needle, ground as finely as possible and under a binocular microscope, minute punctures were made through the epidermis into the subpapillary layer of the corium. The tip of a micropipette, 1/10 mm. in diameter and filled with dye solution, was gently touched to the tissue in the puncture wound. The dye solution filled the cavity by capillarity and without pressure, about 1/20 c. mm. entering in this way, as we have found. From this reservoir the dye spread slowly through the skin just beneath the epidermis. As result, there appeared a colored spot almost circular in form and from 0.8 mm. to 3.3 mm. in diameter. The spreading dye lay in a shallow layer with a smooth marginal outline which remained well defined, as a rule, for more than an hour. A gradual paling took place at the periphery as the dye spread through the tissues, although the margin remained smooth. Gradually too the whole spot of dye became pale. The spots were measured, as will be described below, only while the margins of color were well defined. To indicate that these small pools of dye solution lay interstitially under no pressure and that they were not forcibly injected to form a bleb, they will be termed dye maculae,

for they resembled in shape the common pigment maculae of the skin. The dye maculae could not be made uniform in size, a circumstance that raised certain problems to be discussed below.

In many experiments the small micropuncture wounds ruptured lymphatic capillaries, which the dye solution promptly entered. In other instances, especially during the formation of edema, dye solution oozed out from the maculae and spread upon the surface of the skin. No attempts were made to measure the spread of the maculae under either of these conditions.

Within less than a minute after the dye came in contact with the tissues the outlines of the resulting dye spots were drawn by means of a camera lucida attached to a binocular dissecting microscope. Later the areas of the drawings were determined by a planimeter. Similar measurements and tracings were repeated after half an hour and again after the lapse of an hour. For the sake of simplicity the initial areas are expressed in planimetric units. The relationship of these units to the actual size of the spots will appear below. The spread of the dye has been expressed in terms of the increasing areas of the maculae. For example, if the final area of a dye macula, expressed in planimetric units, was 2½ times as great as its initial area, the spread was called 2.5.

Perfusion Experiments.—To determine the effect of the pulse upon the spread of dye through tissues we perfused amputated ears of rabbits with a pulsatile or non-pulsatile flow of defibrinated rabbit's blood, using for the purpose a technique and apparatus described in the preceding paper (1). The dye solutions were introduced into the perfused ears and the resulting spread measured as just described. All the rabbits used for this work were of about 2000 gm. body weight.

The preceding paper has shown that lymph flow in ears supplied with a pulsating flow of blood is greater than in those perfused at constant pressure, even when both the blood pressure and rate of flow are less. During the present work, when comparing the rate of interstitial dye spread in 17 ears perfused at constant pressure with that occurring in 18 ears supplied with a pulsating flow, the advantage of pressure has been given to the former. For example 9 of the constant pressure perfusions were done at a pressure of 141 mm. of mercury. The pressure in 4 experiments stood at 131 mm., in 3 at 120 mm., and in 1 at 152 mm. of mercury. 6 of the perfusions with pulsatile flow were done at pressures of 141/60 mm. of mercury, 5 at 131/60 mm., 2 at 120/60 mm., 1 at 115/60 mm., 2 at 100/60 mm., and 2 at 95/60 mm. Some of these pressures are higher than those existing in the living animal (8–10), others equal to the normal or lower. Wishing to employ throughout the experiments a diastolic pressure approximately like that in normal rabbits, we selected arbitrarily the pressure of 60 mm. of mercury. In order to obtain sufficient blood flow through the ear to give a normal appearance to the organ under the microscope it was often necessary to employ a "systolic" pressure of 141 mm. of Hg, yet some experiments were done with "systolic" pressures of only 95 or 100 mm. of mercury. As the data below show, the findings

were similar in all the experiments, so the results are not to be attributed to an abnormally high pulse pressure in the experiments involving "systolic" pressures of 141 mm. of mercury. It should be noted that the mean pressure in all the pulsatile perfusion experiments was always far lower than the lowest constant pressure employed. However, in describing the effects of pressure differences upon the findings it has been convenient to employ the figure for the "systolic" pressure of a pulsating flow of blood as if it represented the true pressure. In comparing the results of experiments, for example, in which the "systolic" pulsatile pressure was 120 mm. of mercury and the constant pressure stood at the same figure, the pressures will be called "equal," although as a matter of fact the mean pressure was lower in the instance first mentioned. In cases in which the pulsatile pressure is termed higher than the constant pressure we actually mean that the "systolic" pressure of the former is higher than the latter.

The rate of flow of the perfused blood could be regulated only within small limits, since it varied with the state of dilatation or contraction of the arterial tree of the ear. For example, if the arteries and arterioles of the ear remained in a state of relative contraction the blood flow, whether impelled by pulsatile or constant pressures, remained small. Increased flow could only be attained by an increase in the pressure of the perfusate. But we had determined to keep the pulsatile "systolic" pressures no higher than 141 mm. of mercury. As result, in some experiments the flow of blood was much smaller than in others. In many experiments on the other hand the flow from the perfusion apparatus was too great. This could be reduced at will, as described in the preceding paper (1) by adjusting a screw clamp near the outlet of the perfusion apparatus. The rate of blood flow and the pressures were equal in some experiments, while in others the rates of flow or the pressures of the perfusates, or both, differed greatly. As result, we were able to compare the spread of dye under these varying conditions.

In every case when the final observations had been made, 0.03 cc. to 0.1 cc. of a 21.6 per cent aqueous pontamine sky blue solution, isotonic with blood, was injected through the delivery tube of the perfusion apparatus, to circulate in the vessels of the ear, while the rate of blood flow was measured. The distribution of dye in and about the maculae and the rate at which the color was carried upon the blood to the tissues was noted. Finally the ear, which had been weighed prior to the perfusion, was weighed again, and pieces of tissue removed for section.

During each experiment the appearance of subclinical or of frank edema was carefully sought for. Using a sharp dissecting needle and watching with the binocular microscope, we endeavored to elicit evidence of microscopic pitting upon pressure. In many of the experiments edema occurred and could be recognized in this way. Some ears became intensely edematous, while others showed but the faintest traces of the condition or developed sharply localized edematous patches involving one or more of the dye maculae. All these instances will be considered separately, below.

The Spread of Dye in the Intact Ears of Normal Rabbits

In 8 experiments dye maculae were placed in the ears of normal unanesthetized rabbits sitting quietly in a box, tilted in such a way that the upper surface of the animal's ear containing the dye spots remained horizontal. In each experiment dye was instilled into 6 or 8 regions of the middle and outer thirds of the ear, and the interstitial spread measured as described. Only a few experiments were done, just enough to obtain findings which might serve as a measure of normality for the appraisal of the results obtained in perfusion experiments.

In some of the experiments not included in the 8 mentioned above, the ear under observation became intensely hyperemic. These experiments were ruled out for it was found, in work to be reported later, that in the ear of the mouse the interstitial spread of dye is enhanced by active hyperemia. In other experiments dye appeared in lymphatics or in blood vessels during the periods of observation. These instances too were ruled out, for dye drained away in the vessels instead of spreading interstitially. There remained in the 8 experiments 21 dye maculae that had been placed in ears in which the circulation seemed normal during the experimental period. As already mentioned these dye spots could not be made uniform in size. It was to be expected that large spots would not increase in size as rapidly as small ones. To demonstrate the rate of interstitial dye spread in the normal ear and to rule out the influence of the variations in size of the maculae, we have divided them into groups according to their initial size. The spread of the spots in each group is indicated in Table I, the first group containing maculae less than 50 planimetric units in initial area, that is to say spots originally 0.8 to 1.0 mm. in diameter, the second group containing spots 50 to 100 planimetric units in original area, 1.1 to 1.5 mm. in diameter. The next group included the maculae of 100 to 200 and the last those of 200 to 300 planimetric units, spots varying roughly from 1.7 mm. to 2.4 mm. and from 2.5 to 3.3 mm. in diameter. The data for each group are arranged in three vertical columns, the first showing the initial area of each spot, the smallest at the top, the largest at the bottom, the second and third columns showing the spread of the spots after half an hour and after one hour respectively. As already described the spread is expressed as the number of times the spot increased its initial area. For example in the first group a spot 23 planimetric units in area became 115.5 planimetric units in area after half an hour and 174.4 units after an hour, thereby increasing its initial area 5.0 and 7.6 times. The averages of the initial areas and of the increases in size of each group are given at the bottom of each column.

Inspection of the averages given in Table I shows that, as one would expect, the smaller dye spots increased in size more rapidly than the larger ones. Spots of approximately equal initial size spread more

uniformly. Hence to compare the dye spread in ears perfused with pulsating blood with that taking place in ears perfused with a constant flow we have used only the data from groups of spots having approximately the same initial size.

TABLE I

The Spread of Dye Spots of Differing Sizes in the Ears of Normal Rabbits

Initial area less than 50 planimetric units			Initial area between 50 and 100 planimetric units			Initial area between 100 and 200 planimetric units			Initial area between 200 and 300 planimetric units		
Initial areas	Interstitial spread of dye		Initial areas	Interstitial spread of dye		Initial areas	Interstitial spread of dye		Initial areas	Interstitial spread of dye	
Planimetric units	Ratio* of spread		Planimetric units	Ratio of spread		Planimetric units	Ratio of spread		Planimetric units	Ratio of spread	
	After ½ hr.	After 1 hr.		After ½ hr.	After 1 hr.		After ½ hr.	After 1 hr.		After ½ hr.	After 1 hr.
23	5.0	7.6	52	3.9	6.6	101	4.7	8.5	287	2.7	3.4
29	5.2	9.0	59	4.0	8.1	107	3.1	4.9			
39	3.4	6.8	63	3.5	6.4	115	2.8	4.9			
42	4.6	6.1	77	5.9	5.6	120	3.3	4.3			
43	5.2	9.6	78	3.1	5.8	142	2.6	4.6			
			96	4.8	6.5	159	5.2	7.9			
						163	3.5	5.9			
						182	3.6	5.1			
						198	2.5	3.4			
Average..35	4.7	7.8	78	4.2	6.5	143	3.5	5.5			

* See text and legend.

The table compares the interstitial spread of dye spots of differing sizes in the ears of normal rabbits. The spots have been classified in 4 groups according to their size as measured in planimetric units (see text). The data from each group have been arranged in three vertical columns, the first showing the initial area of each dye spot, the smallest at the top, the largest at the bottom. The second and third columns in each group represent the spread of the spots after ½ hour and 1 hour respectively. The spread has been presented as the ratio of the area of a given dye spot after 30, or 60, minutes to its initial area. For example, when it was found that the area of the first spot in group 1, after 30 minutes, was 5 times larger than at the beginning of the experiment, the spread was expressed as 5.0.

It will be noted that the smaller spots spread more rapidly than the larger ones.

The Spread of Dye in Amputated and Perfused Rabbit Ears

The Spread of Dyes through Edema-Free Tissues.—In 49 experiments the ears of rabbits of about 2 kilos were amputated and perfused with

defibrinated rabbit's blood, as described in the preceding paper. During the perfusions, 6 or 8 dye maculae were placed in each ear, in all 274 of them, 129 in 24 ears perfused with pulsatile flow and 145 in 25 ears perfused at constant pressures. Of the 49 experiments 35 were free from objection on the scores already mentioned and in these the rate of enlargement of 184 dye spots was measured for periods of an hour, 72 spots in 18 ears perfused with pulsating blood and 112 in 17 ears perfused at constant pressure.

The spread of these spots has been considered after separating the data into comparable groups in relation to the initial size of the dye spots, the pressure and the rate of blood flow per gram of ear, and the occurrence of local or general edema. In about 30 per cent of the experiments local or general edema appeared in the perfused ears and was demonstrated under the binocular microscope by pressure with a dissecting needle. The findings in these experiments will be described separately below.

The data showing the spread of the dye spots in edema-free tissue have been graphically depicted in Chart 1 by plotting the average spread of groups of similar sized dye spots along the ordinate and their initial measurements along the abscissa. As already done in the experiments on normal animals, the spots in both pulsatile and in constant pressure experiments were divided into groups according to their original size. The first group included spots up to 50 planimetric units in initial area (0.8 to 1.0 mm. in diameter), the second group those between 50 and 100 (1.1 to 1.5 mm. in diameter), the next 3 groups, those of 100 to 200, 200 to 300, and 300 to 400 planimetric units. In the experiments employing pulsatile perfusion, the spots of dye initially less than 50 planimetric units in area averaged 24 units in area. After spreading for one hour, the areas had increased on the average 8.2 times. To represent this fact in the chart, a point (marked with a cross, x) was located 24 units along the scale of the abscissa and 8.2 units along the scale of the ordinate. Spots of dye originally more than 50 and less than 100 planimetric units, averaging 74 units, increased in area on the average 5.9 times. The curve therefore runs through the next point, 74 on the abscissa, 5.9 on the ordinate. In this way each group's average of initial size and final spread has been plotted. Similarly the averages of the initial areas and the spread of the groups of spots in ears perfused at constant pressure have been plotted in small black circles (●). To compare the spread of the dye spots in ears perfused with pulsatile and with constant currents, we have charted the heavy, continuous line, marked P, representing the findings in the ears perfused with pulsatile current, and the light, continuous curved line, C, representing those obtained in the constant pressure experiments. Both lines show that in both types of perfusion the smaller

maculae spread relatively faster than larger ones. They show further the fact that, in each group of similar initial size, spread was always greater in the ears perfused with pulsating blood. For comparison we have also plotted in the

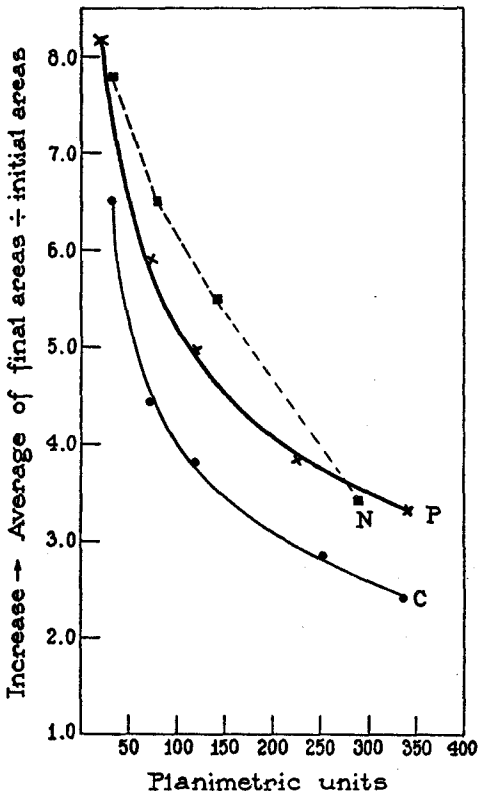

CHART 1. *The Interstitial Spread of Dye Spots of Differing Sizes in the Ears of Normal Rabbits and in Amputated Ears Supplied either with a Pulsatile Flow of Blood, or with a Constant Flow.*—The heavy continuous line, P, represents the spread of dye maculae in ears perfused with a pulsating flow of blood. The fine continuous line, C, shows the spread of maculae in ears perfused with blood at constant pressure. The dotted line, N, shows the spread of dye spots in the ears of normal rabbits. The manner in which the curves are derived is fully described in the text.

same manner on Chart 1 the data from Table I, showing by small squares (■) the averages of the initial areas and the spread of each of the four groups of dye spots in that table. The resulting dotted line, N, connecting these squares represents the spread of the groups of similar sized dye maculae in the ears of normal

rabbits. Spread of dye in the normal ear was slightly greater than in the amputated ears perfused with pulsatile blood flow but the difference was not marked.

A table like Table I was constructed to show the spread of individual spots of the various groups of maculae in the amputated ears. This has not been included in the paper, but it should be noted that the spread of dye spots in the perfusion experiments was far more regular than in the normal ears. This difference is due, no doubt, to

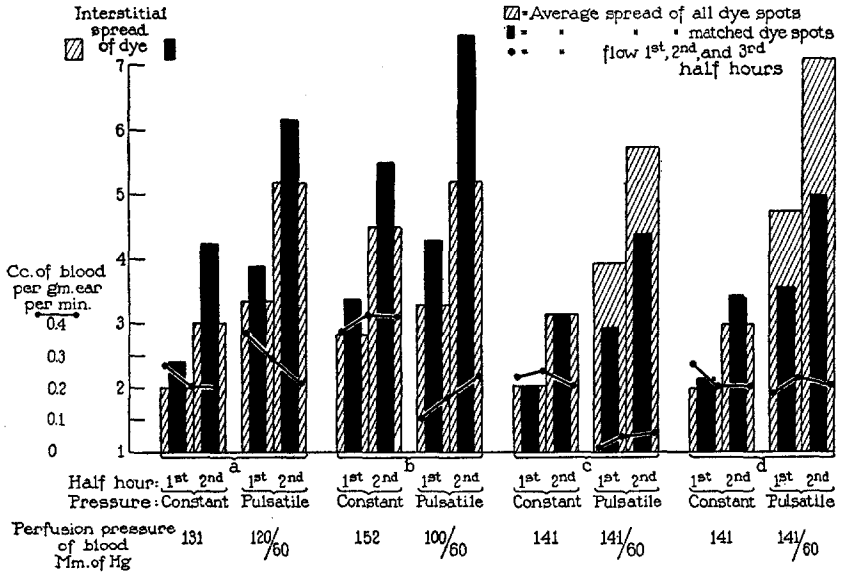

CHART 2. *The Effect of the Pulse on the Interstitial Spread of Dye.*—The chart is fully described in the text.

the fact that the conditions of blood flow and pressure in the normal ears varied much more than did the conditions of flow and pressure in the perfused ears.

Chart 1 shows that the relative rate of spread of the dye spots varied inversely as their size in both types of experiment and that, on the average, maculae of approximately the same size spread more under the influence of pulsation than in its absence. From the chart it is clear, too, that were one to make maculae of dye all under 50 planimetric units (0.8 to 1.0 mm. in diameter), and to compare their

spread exclusively with large ones, for example 300 to 400 planimetric units in area, over 3.3 mm. in diameter, the influence of size would affect their relative rate of increase to such an extent that the physiological difference under study would not be apparent. Actually in performing the experiments one never made all large or all small maculae: inevitably the spots varied much in size. From the findings to be described below it will be obvious too that a fairly even distribution of maculae of the different sizes occurred in both kinds of experiment. The average spread of all maculae of various sizes in all the ears perfused with pulsating blood, with rapid or slow flow, was 6.1 times the average of their initial areas. Maculae in ears perfused at constant pressures spread on the average 4.1 times. Yet the average rate of blood flow in the constant pressure perfusions was 150 per cent greater than in the pulsatile perfusions.

To bring out the important influence of a pulsatile current we have plotted in Chart 2 the data from 8 typical experiments grouped as 4 sets, *a*, *b*, *c*, and *d*, each of which represents the measurements taken in one ear perfused with a constant flow of blood and in another served with a pulsating current of blood. These 8 experiments were selected for comparison, and paired as shown for the reason that in each pair of ears there could be found at least 3, or more, maculae of approximately the same size, while at the same time, in the same ears, other maculae were present which varied greatly in size.

In Chart 2 each set of four broad cross-hatched columns with narrow solid black columns included within them represents the data from one of the comparisons as described above. The data from the constant pressure perfusion stand in the first two columns at the left in each set of four, those from the pulsatile pressure perfusion, to be compared with it, in the two columns at the right. The first and second of the broad, cross-hatched columns in each set of four show the average spread for the first and second half hours respectively of all dye maculae, usually 6 or 8 of various sizes placed in the ear perfused with constant flow. The third and fourth broad columns in each set represent the same findings in the ear perfused with pulsating blood. All the broad, cross-hatched columns represent the spread of dye spots unmatched in size. We have contrasted with this, by means of the narrow black columns, the spread of dye spots of approximately the same initial size in the same ears perfused at constant and pulsatile pressures, selecting for comparison, experiments in which we were able to pick out from each ear at least 3, and sometimes even 6 pairs of spots, all of which were practically the same in initial size. As just mentioned the narrow columns

represent the average spread of these matched pairs of spots. The first narrow solid black column represents their spread in the first half hour, the second, for the next similar period. The rate and the variations in the flow of blood per gram of ear tissue have been shown by the heavy continuous lines joining black dots. The latter represent the upper and lower limits of the variations in flow during each half hour period during which the spread of dye was measured. The lines therefore indicate roughly the rate of flow during each period. The pressures employed for each constant pressure experiment, as indicated, were always as high or higher than the "systolic" pressure in the pulsatile perfusions.

TABLE II

The Spread of Dye Spots of Approximately Equal Size in the Perfused Ears of Rabbits

Perfused at constant pressure			Perfused at pulsating pressure			Non-perfused ears of dead animals		
Initial area	Interstitial spread of dye		Initial area	Interstitial spread of dye		Initial area	Interstitial spread of dye	
Planimetric units	Ratio* of spread		Planimetric units	Ratio of spread		Planimetric units	Ratio of spread	
	After ½ hr.	After 1 hr.		After ½ hr.	After 1 hr.		After ½ hr.	After 1 hr.
109	2.2	3.2	111	2.8	4.5	100	2.0	2.6
110	2.6	3.3	112	2.7	4.9	111	1.9	2.5
111	2.5	3.5	118	3.6	5.0	112	1.9	2.6
112	3.3	5.0	119	4.3	8.7	117	1.9	2.7
112	2.6	3.9	119	2.4	4.8	120	1.9	2.6
114	2.2	3.6	121	2.8	4.2	122	1.8	2.5
120	2.9	3.8	123	2.6	3.6	123	2.1	2.5
124	2.4	3.0	124	3.0	4.5	124	1.9	2.6
Average..114	2.6	3.7	118	3.0	5.0	116	1.9	2.6

* The table is fully described in the text.

In every comparison the unmatched dye spots spread farther when pulsatile currents were used. The spread of the matched spots of equal size showed still greater differences. As the experiments were selected in order to compare the spread of dye spots of equal size, the differences in rate of blood flow and pressure during the perfusions varied greatly. Some consistent findings resulted, for example in Chart 2 *d*, the blood flow in each ear was about equal all through both experiments and the pressures were 141 mm. and 141/60 mm. of mercury. At equal pressures and equal rates of flow the spread of

the unmatched dye spots (cross-hatched columns) and also that of the spots matched in size (narrow black columns) was greater in the ear perfused at pulsatory pressure. In the pairs of experiments shown in *b* and *c* the flow during the pulsatile pressure perfusions was small compared with that of the constant pressure perfusions, as shown by the lines crossing the columns. In *c* the pressures were "equal," being 141 and 141/60 mm. of mercury, in *b* the pulsatory pressure was lower than the constant pressure. Nevertheless the spread of both matched and unmatched dye spots was greater in the pulsatile perfusion experiments. In the pair of experiments compared in Chart 2 *a*, the blood flow during the pulsatile perfusion was greater than that during the constant pressure perfusion but the pulsatory pressure of the former, 120/60 mm. of mercury, was lower than that of the constant pressure experiment, 131 mm. of mercury. Dye spread was greater in the pulsatile perfusion experiment.

It is clear from these findings that the effect of pulsation upon the spread of dye solution was sufficiently great to overcome all the other variable influences, such as differences in size of the dye spots, rate of perfusion flow or its pressure.

To show the effect of pulsation upon the interstitial spread of dye, we have compared in the first two sections of Table II the spread of 16 dye spots, 8 from ears perfused with pulsating blood and 8 from ears perfused with constant flow. All had initial areas of more than 100 and less than 125 planimetric units, that is to say the spots were almost the same size, varying only from 1.7 to 2.0 mm. in diameter. The particular limits, 100 to 125 planimetric units, were selected because the differences in size were small enough to yield maculae of comparable area, and more maculae happened to fall within these limits than in those of any other similarly narrow range. All the spots had been placed in ears that remained free from edema, and the experiments were free from technical errors. A comparison of the spread of these dye spots has been made in the table without regard for the differences in the rates of flow of the perfused blood or its pressure. For comparison the table shows also the spread of 8 maculae of similar size introduced into the non-amputated ears of rabbits 2 to 3 minutes after they had been killed with ether. In the latter the spread of dye was about two-thirds as great, at the end of one

hour, as in the ears perfused at constant pressure, and about half as great as in the ears perfused at pulsating pressure.

In a few instances we were able to compare the spread of dye maculae of equal initial area in ears which happened to be perfused with equal volumes of blood per gram of tissue, and at "equal" constant

TABLE III

*A Comparison of the Spread of Dye Maculae of Nearly the Same Size in Ears Perfused with a Constant or a Pulsating Flow of Blood at Nearly Equal Rates of Flow and at Equal Pressures**

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(1a)	(2a)	(3a)	(4a)	(5a)	(6a)	(7a)
Exp. No.	Initial area	Pressure of perfusate	Rate of blood flow per gram of ear per minute		Ratio of spread		Exp. No.	Initial area	Pressure of perfusate	Rate of blood flow per gram of ear per minute		Ratio of spread	
	Planimetric units	mm. Hg	In first ½ hr.	In second ½ hr.	After ½ hr.	After 1 hr.		Planimetric units	mm. Hg	In first ½ hr.	In second ½ hr.	After ½ hr.	After 1 hr.
I	23	141	0.29	0.28	3.8	5.1	1	25	141/60	0.25	0.24	8.4	13.0
II	48	120	0.19	0.23	1.7	3.2	2	52	120/60	0.20	0.21	4.0	6.3
III	64	131	0.28	0.30	2.2	3.7	3	64	131/60	0.23	0.28	4.1	6.0
							4	66	131/60	0.25	0.22	4.2	5.8
IV	70	141	0.26	0.21	2.4	4.2	5	74	141/60	0.22	0.28	3.8	5.2
V	87	141	0.24	0.23	1.9	3.7	6	93	141/60	0.27	0.30	3.3	5.4
VI	96	120	0.18	0.20	2.0	3.6	7	99	120/60	0.16	0.19	3.7	5.9
VII	116	141	0.28	0.30	2.9	3.9	8	116	141/60	0.35	0.24	2.4	4.8
							9	124	141/60	0.28	0.33	3.0	4.5

The table, fully described in the text, shows that dye spread more rapidly in tissues perfused with a pulsatile flow of blood than in those perfused at constant pressure.

* "Systolic" pressure of the pulsatile perfusion equal to constant pressure.

and pulsatile pressures, that is to say, at a constant pressure equal to the "systolic" pressure of the pulsating perfusate (Table III). In some instances the spots compared were of slightly unequal size, and in such instances we selected from the constant pressure perfusions only those spots which were smaller than those from the pulsatile

perfusions with which they were matched. The former should have spread relatively faster than the latter, other things being equal. Only a few comparisons were possible under these rigid conditions. The spots used for Chart 2 *d* have not been included in the table.

Columns 1 to 7, placed to the left of the double lines in the center of Table III, show the data obtained from 7 spots of dye in ears perfused at constant pressure. In the vertical column 1 we have given distinguishing Roman numbers to each dye spot. The vertical columns 2 to 7 show, respectively, the initial areas of these maculae in planimetric units, the pressure of the perfusate, the rate of blood flow per gram of ear in the half hour periods of the experiment, and the spread of the dye spots during these periods. The data concerning these spots have been compared in the right half of the table, columns 1*a* to 7*a*, with similar data on the spread of 9 dye spots found in ears perfused with pulsating blood, the "systolic" pressure of which equalled the pressure employed in the perfusions at constant pressure, while the rate of blood flow differed but little. Column 1 *a* contains distinguishing numbers for these spots. Between each set of horizontal spaces appear the comparable data, that is to say, the spread of each spot in an ear perfused at constant pressure is compared with that of one spot, or occasionally two spots, of almost equal size in ears perfused at pulsatile pressure. For example, comparing the spread of dye spot III with that of spots 3 and 4, the constant pressure, column 3, equalled the "systolic" pressure in the experiments with pulsating current, from which the 2 spots were taken, column 3*a*. The flow of blood in the constant pressure perfusion, columns 4 and 5, was slightly greater than the flow in both of the pulsatile pressure perfusions, columns 4*a* and 5*a*. Dye spot 3 in an ear perfused with pulsating current, spread 4.1 times its initial area in half an hour, and 6.0 times in an hour, columns 6*a* and 7*a*. Dye spot 4 spread 4.2 and 5.8 times in similar periods. These figures are to be compared with those in columns 6 and 7 respectively, showing the spread of the dye spot in the ear perfused with a constant flow of blood, during equal time intervals. In a similar manner the other comparisons can be made.

The table shows that dye spread more rapidly in the tissues perfused with a pulsatile flow of blood than in tissue perfused at constant pressure, when the rates of flow were equal and the "systolic" pressure of the pulsatile perfusion equalled the constant pressure of the compared experiment.

The Effect of Variations in the Flow of the Perfused Blood.—As already mentioned, the rate of flow of the perfused blood varied much from one experiment to another, at times by accident, at times by design. Nevertheless in the ears perfused with pulsatile flow, dye spread more rapidly than in those perfused with a constant current,

TABLE IV

The Spread of Dye Maculae of Nearly the Same Size in Ears Perfused with a Constant or a Pulsatile Flow of Blood at "Equal" Pressures but at Greatly Differing Rates of Flow

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(1a)	(2a)	(3a)	(4a)	(5a)	(6a)	(7a)
Exp. No.	Initial area	Pressure of perfusate	Rate of blood flow per gram of ear per minute		Ratio of spread		Exp. No.	Initial area	Pressure of perfusate	Rate of blood flow per gram of ear per minute		Ratio of spread	
	Planimetric units	mm. Hg	In first ½ hr.	In second ½ hr.	After ½ hr.	After 1 hr.		Planimetric units	mm. Hg	In first ½ hr.	In second ½ hr.	After ½ hr.	After 1 hr.
I	19	131	0.30	0.26	3.6	5.1	1	25	131/60	0.21	0.22	6.3	9.2
II	64	120	0.26	0.32	2.3	3.8	2	68	120/60	0.22	0.19	4.4	6.4
III	74	141	0.29	0.28	2.1	3.6	3	76	141/60	0.16	0.13	4.0	6.2
							4	79	"	0.12	0.13	4.2	6.3
IV	93	141	0.31	0.32	2.4	4.0	5	94	141/60	0.19	0.20	3.8	5.2
							6	103	"	0.05	0.06	3.8	5.6
V	134	141	0.11	0.13	2.8	3.7	7	133	141/60	0.08	0.11	4.3	6.3
							8	143	"	0.05	0.06	3.8	5.6
VI	73	141	0.21	0.22	2.6	3.6	9	77	141/60	0.41	0.23	3.4	5.4
							10	79	"	0.23	0.28	4.4	6.6
VII	77	120	0.18	0.23	2.1	3.6	11	83	120/60	0.23	0.28	3.7	5.6
							12	84	"	0.27	0.30	3.9	5.8
VIII	77	131	0.11	0.13	2.3	3.5	13	79	131/60	0.31	0.33	4.4	6.4
							14	81	"	0.23	0.28	3.8	6.0
IX	108	141	0.19	0.18	2.5	3.3	15	114	141/60	0.22	0.24	4.1	5.2
							16	116	"	0.35	0.23	2.4	4.8
							17	124	"	0.28	0.33	3.0	4.5
X	109	131	0.18	0.21	2.7	3.8	18	124	131/60	0.25	0.21	3.4	5.8
							19	126	"	0.22	0.23	4.0	6.5
XI	116	141	0.28	0.30	2.1	3.8	16	116	141/60	0.35	0.23	2.4	4.8
							17	124	"	0.28	0.33	3.0	4.5
XII	126	131	0.12	0.13	2.4	3.0	18	124	131/60	0.25	0.21	3.4	5.8
							19	126	"	0.22	0.23	4.0	6.5

The table, as described in the text, shows that dye spread more rapidly in the ears perfused with a pulsating current of blood, in spite of great differences in the rate of blood flow. The pressures were equal.

even when the flow in the former was but one-quarter that in the latter. To illustrate this fact further, we have presented in Table IV data showing the spread, in groups, of all the dye spots of almost the same size that could be found in experiments in which the constant pressures and "systolic" pulsating pressures were equal, but in which the rates of flow differed greatly. The number of maculae that fulfilled these conditions and could be compared was limited; the table contains the data from all of them, 12 from constant pressure experiments and 19 from pulsatile flow perfusions.

The plan of the table is like the preceding one. In each section of the left side of the table and between the horizontal spaces appear the data on the spread of single dye spots in ears perfused at constant pressure. They are to be compared with the data in the right half of the table lying between the same horizontal spaces. These figures give the data concerning the spread of those maculae comparable in initial size, or very slightly larger, which were found in ears perfused at pulsatile pressures (the "systolic" impulse of which equalled the constant pressure) but with differing volumes of blood per gram of tissue per minute. The first section of the table compares the spread of maculae during perfusions in which the pulsatile flow was less than the flow at constant pressure. The second section shows the spread when the pulsatile flow was greater. In the table, the data from certain dye spots reappear from time to time and can be recognized by the distinguishing number given to each spot.

In all these comparisons the spread of dye spots in constant pressure experiments has been compared as usual with that of equally large or slightly larger spots from pulsatile pressure experiments. The advantage is therefore given to the constant pressure experiments as already described. A glance at the figures in columns 6 *a* and 7 *a*, comparing them with the figures in columns 6 and 7, shows that the maculae in the pulsatile perfusion experiments spread more rapidly than those in the constant pressure perfusions regardless of the differences in the rate of blood flow. For example, in the comparison of the spread of dye spots IV and 6, the volume of flow of the pulsating perfusate per gram of ear was but 1/6 that in the constant pressure perfusion with which comparison was made; nevertheless dye spread was much greater in the former.

The Effects of Variations in Pressure.—The findings so far reported show further that the greater dye spread in ears perfused with a pulsatile blood flow cannot have been due to the pressure of the latter.

For example, the comparison in Chart 2 *b* shows a greater dye spread in an ear perfused with a pulsatile flow than in one perfused with a constant flow although both the pressure and the rate of flow in the former were lower than in the latter. In this connection there is one comparison which can be made from our data which is of additional interest. A perfusion, done at a pulsatory pressure of 95/60 mm. of mercury, resulted in the circulation of only 0.11 cc. and 0.12 cc. of blood per gm. of ear per minute, in the first and second half hour

TABLE V

The Spread of Dye Maculae of Nearly the Same Size in One Ear Perfused with a Constant Flow of Blood and in Another Ear Perfused with a Pulsatile Flow of Blood. Equal Rates of Flow but Different Pressures Were Used

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(1a)	(2a)	(3a)	(4a)	(5a)	(6a)	(7a)
All spots in the same ear	Initial area	Pressure of perfusate	Rate of blood flow per gram of ear per minute		Ratio of spread		Both spots in the same ear	Initial area	Pressure of perfusate	Rate of blood flow per gram of ear per minute		Ratio of spread	
	Planimetric units	mm. Hg	In first ½ hr.	In second ½ hr.	After ½ hr.	After 1 hr.		Planimetric units	mm. Hg	In first ½ hr.	In second ½ hr.	After ½ hr.	After 1 hr.
	74	141	0.14	0.12	2.8	3.7		61	95/60	0.11	0.12	4.3	6.3
	78	141	0.14	0.12	2.8	3.7							
	111	141	0.14	0.12	2.5	3.5		116	95/60	0.11	0.12	4.3	8.7
	110	141	0.14	0.12	2.6	3.3							

This table, described in the text, gives the data from 2 experiments in which it was possible to compare the spread of dye in one ear perfused with a pulsatile current of blood at a low pressure, with the spread of dye in another ear perfused at a higher constant pressure but with an equal volume of blood per gram of tissue per minute. The spread of dye was greater in the ear supplied with a pulsatile flow of blood.

periods, respectively. We are able to find one experiment done with constant pressure in which the perfusion rate was comparable, but in which a higher perfusion pressure, 141 mm. of mercury, had been used. Fortunately, 4 of the dye spots in the latter compared closely in size with 2 in the former. The data concerning their spread are shown in Table V. Despite the lower pulsatile pressure, dye spread was greater in the ear perfused in this way. It is to be noted too that in this experiment the pulse pressure was small.

The Effect of Edema

So far the findings in only two-thirds of our experiments have been considered, for in the other third edema appeared in the tissues of the perfused ears despite all efforts to avoid it. Edema occurred more often in the experiments involving constant pressure perfusion than in those done with pulsations. It appeared frequently in the perfusions done at the highest pressures and with the greatest flow of blood, but this finding was by no means regular. No single group of circumstances could be found which seemed to favor its appearance. We took the opportunity to study the spread of dye during the formation of edema in the amputated ears while they were perfused with a constant or pulsatile flow of blood. The studies were made as already described save that the maculae of dye were placed in regions of the ears already edematous and becoming more so, or in skin that happened to become edematous a few minutes later. The findings need no detailed report. In 13 perfused ears in which edema appeared, more than 95 maculae had been made. The spread of only 77 of these was measured, 36 from pulsatile perfusions and 41 from those done at constant pressures. The remainder were ruled out because the dye solution together with edema fluid oozed out of the tissue on the surface of the skin.

In Chart 3 we have plotted the spread of these maculae in the same manner as in Chart 1, dividing the spots into 5 groups according to their initial sizes.

The heavy continuous line, PE, represents the spread of dye in the ears which became edematous during the pulsatile perfusions, the heavy dotted line, CE, the dye spread during the perfusions at constant pressure. We have reproduced in the same chart the curves from Chart 1 representing the dye spread in non-edematous ears, the light continuous line, P, showing that which occurred during the pulsatile perfusions, the light dotted line, C, that taking place during perfusions at constant pressure.

As the chart shows, dye spread most in the ears which became edematous during perfusion with a pulsatile flow of blood, line PE. Spread was slightly greater than in ears similarly perfused but not edematous, line P. By contrast the positions of lines CE and C, in the chart, demonstrate an important fact; the onset of edema adds but little to the rate of dye spread in ears perfused with constant

pressure. Indeed the spread of dye in these edematous ears was less than that occurring in non-edematous ears perfused with a pulsatile flow of blood. More will be said of this below. It is to be stressed that our work has allowed us to study the dye spread only during the formation of edema, not after its formation. In work to be

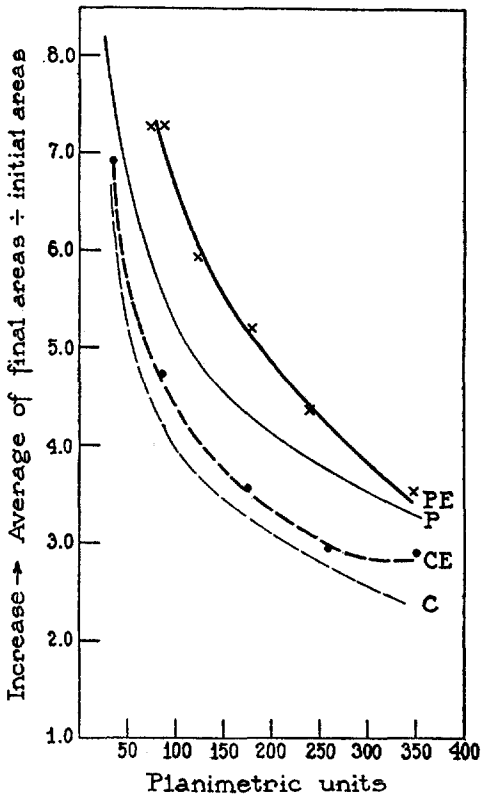

CHART 3. *The Spread of Dye Spots in Ears Becoming Edematous While Perfused with a Pulsatile or a Constant Flow of Blood.*—The chart is described in the text.

published it has been shown that dyes introduced into the connective tissue in the same way as in the present experiments spread farther in tissues becoming edematous than in those already boggy. Similar findings would doubtless have appeared had a comparison been made in the course of the present work.

The Effect of Pulsation of the Vessels upon the Removal of Dye from the Tissues

It was frequently noticed, toward the end of the hour during which the measurements were made, that the dye maculae lost some of their color, not only near their margins but throughout. This occurred chiefly in the tissues perfused at pulsatile pressure. In these instances, too, it was usually noted that the apparent spread of the dye through the tissues was far less in the second half hour than in the first, and when, upon a few occasions, measurements were continued for a third half hour period, the spot of color often became smaller as well as paler. Obviously the dye was being rapidly removed from the tissues. These phenomena appeared usually in the pulsatile perfusion experiments and seldom, and then only dubiously, in the constant pressure perfusions. It seemed wise, therefore, to test the effect of the pulse upon the rate of absorption of dye from the tissues. To do this a total of 48 maculae were made in the usual manner in 14 of the experiments described above, employing a highly diffusible dye, patent blue V, which we have used in many previous studies of the physiology of lymphatics of animals (5–7) and of man (2–4). The spread of these maculae was observed and measured as already described. A constant pressure of 141 mm. of mercury and a pulsatile pressure of 141/60 mm. of mercury were selected for the experiments. In all those from which data are considered here the blood flow was greater when constant pressures were used. The diffusible dye spread with great rapidity so that the spots often became too pale to measure accurately after an hour. Furthermore, edema appeared in the ears in about one-third of these experiments. As result, in only 24 instances could the spread of these maculae be measured accurately for periods up to one hour in ears which did not become edematous. 13 of these dye spots were made in ears perfused at constant pressure and only 11 in the pulsatile perfusion experiments. Nevertheless these scanty data showed such a constant difference that further experimentation was considered unnecessary.

In Table VI the data have been arranged as in Table I to show the spread of the dye spots after 30 minutes and after 1 hour. When pulsatile perfusion was done the average of the areas of the 11 maculae, varying in original size from 77 to 180

planimetric units, increased 5.9 times within 30 minutes. In the next similar period, however, the average of their areas decreased, becoming only 4.3 times the average of the initial areas. Of the 13 maculae, in ears perfused at constant pressure, the initial areas of 8 fell within the limits of the initial areas of the maculae in the ears perfused with pulsation. The data gathered from these instances are enclosed in the box of heavy lines in the table. The average of the areas of these 8 maculae was found to be 4.4 times that of the average of their

TABLE VI

The Spread of Maculae of a Highly Diffusible Dye, Patent Blue V, in Ears Perfused with a Pulsatile or a Constant Flow of Blood

Perfused at pulsatile pressure			Perfused at constant pressure				
Initial area	Interstitial spread of dye		Initial area	Interstitial spread of dye			
Planimetric units	Ratio of spread		Planimetric units	Ratio of spread			
	After ½ hr.	After 1 hr.		After ½ hr.		After 1 hr.	
77	7.4	7.4	68	5.1		6.6	
84	5.0	4.9	82	4.7		6.9	
87	6.0	3.9	85	5.0		7.5	
97	5.3	2.4	94	5.2		7.3	
113	6.5	4.0	99	4.7	Average, 4.4	7.0	Average, 6.4
121	5.6	4.1	113	5.2		5.0	
135	6.1	4.8	132	3.0		5.4	
151	7.1	5.0	166	5.0		6.2	
155	5.8	2.9	178	6.1		5.8	
173	4.5	3.9	188	5.2		7.0	
180	6.2	4.0	213	3.3		4.5	
			275	4.4		5.0	
			370	2.6		2.8	
Average.....	5.9	4.3		4.6		5.9	

The table is fully described in the text.

initial areas after spreading for ½ hour. During the second half hour of the experiments the average increased to 6.4 times the initial average figure.

An average of the spread of all the maculae in these constant pressure experiments, regardless of whether the initial areas were greater or smaller than those of the maculae in the pulsatile perfusion experiments, also shows, during the second half of the experiments, a continued increase in size from 4.6 to 5.9 times the average of their initial areas. The dye spots in the constant pressure experiments did not decrease in size as did the maculae of the pulsation perfusions.

These measurements do not adequately portray the fact which was clearly recognizable to the observer, that dye absorption was far more rapid in the ears perfused with a pulsating flow of blood. As already mentioned many of the maculae watched under such conditions became too pale to measure, and all were far paler at the end of the experiments than the dye spots in the ears perfused at constant pressure. Clearly the pulsation of the blood vessels enhanced dye removal from the tissues as well as spread.

DISCUSSION

It is recognized that the movement of dyes or of dye-colored solutions through a tissue may not be representative of the movement of other substances or fluids in the same tissue. Nevertheless, in the absence of better means, it has seemed important to determine the effect of the pulse upon the interstitial spread of certain dyes. The findings reported here have shown that the pulsation of blood vessels in the ear of the rabbit increases the spread of dye through the skin of the organ after its introduction into the connective tissue.

What can be said of the effect of the pulse upon the interstitial movement of those substances and fluids which are concerned with tissue nutrition? The preceding paper (1) has shown that the pulsation of blood vessels increases the formation and the flow of lymph, that is to say increases the movement of fluid through the tissues. Pulsation also increases the interstitial spread of dyes. One might assume that the pulsation of blood brings about a greater escape of fluid or material from the blood to the tissues, each impact of the pulsation pushing blood constituents against those already passing from the vessel and present in its wall. In this way, particles, large molecules, or fluids might be forced into the tissues like grain thrown repeatedly against a sieve which would let but little by if the grain were merely pressed against it. Materials already present outside the vessel wall, receiving the impact, would be forced to move through the tissues. It should be noted, however, that the effect of the pulse is negligible or absent in those vessels in which the permeability is greatest, namely, the capillaries, and pulsations are strong only in the relatively thick-walled arterioles and arteries. Some other explanation of our results seems to be called for.

Several findings in this and in the preceding work bear directly upon this problem. Experiments involving pulsatile perfusion with but 1/6 or 1/5 the flow and far lower pressures than those used in constant pressure perfusions invariably yielded both greater lymph flow and greater interstitial dye spread. Can one suppose that fluid escape from the blood vessels was greater under the former than under the latter condition? Further, the effect of pulsation in spreading dye and increasing lymph flow is not due wholly to an increase in the fluid content of the tissues. As already stated, in non-edematous ears the formation and flow of lymph was much increased by pulsation (1). When edema was present in the tissues lymph flow and formation were not greatly increased unless there was pulsation. When edema was present in a tissue perfused with a pulsatile flow lymph formation was greatest. It would seem from this that the movement of fluid through the tissues to form lymph required the mechanical effect of the pulse, the mere presence of excess interstitial fluid failing to bring about this result. In this relation it should be mentioned that, in the present work, edema appeared more frequently in ears perfused at constant pressure than in those supplied by pulsating blood; under the former circumstances the gain in weight was greater than under the latter. Whether more fluid escaped from the vessels in the constant pressure perfusions we cannot definitely say, though it seems unlikely. Certain it is that the return of fluid from the tissues to the blood or lymph was aided by pulsation. The more rapid absorption of dye from the tissues supplied by a pulsatile blood flow corroborated this finding.

From the data of the present paper we can infer that, just as the mechanical influence of the pulse is more important than the changes in the fluid content of the tissues in producing lymph flow, so is it more important, as just discussed in the preceding paragraph, in producing interstitial dye spread. The same conditions which increased one increased the other. For example, in non-edematous ears dye spread was much increased by pulsation; when edema was already present dye spread was not greatly increased unless there was pulsation; when excess fluid was present in a tissue perfused with a pulsatile flow, dye spread was greatest.

How can these findings be explained? Other work from this laboratory now in press, demonstrates the importance of mechanical factors in the interstitial movement of dye in the ear of the living mouse. It becomes far more rapid when the tissue is subjected to gentle intermittent changes in external pressure, changes far smaller than those occurring in the blood vessels and equivalent to those produced by columns of water only 2 to 8 cm. in height. These slight changes in pressure applied to the skin of the ear and not from within the vessels as in the present work, spread dye through the tissues with great rapidity. From this it is clear that to explain our findings one need not invoke the action of the pulse within a vessel forcing materials through its walls. If slight changes in pressure applied externally to the tissues spread dye through them rapidly it seems possible that the change in caliber of vessels within a tissue can produce a similar effect. A discussion of the probable mechanism by which the pulse increases the formation and flow of lymph and the spread of dye in the tissues must be postponed for following papers in which further data will be presented concerning the spread of substances through tissues. Suffice it to state that our experiments show that pulsation of the blood vessels in the perfused rabbit's ear leads to greater formation and flow of lymph, to greater interstitial spread of dye, and to the more rapid absorption of dye from the tissues.

SUMMARY

The pulsation of blood vessels in the ear of the rabbit greatly increases the rate of the spread of dye introduced into the subcutaneous tissue.

The appearance of edema in tissues perfused at a constant pressure leads to very little increase in the rate of dye spread. By contrast, a rapid interstitial spread of dye occurs in tissues becoming edematous while perfused with a pulsatile flow of blood.

The significance of these facts is discussed.

BIBLIOGRAPHY

1. Parsons, R. J., and McMaster, P.D., *J. Exp. Med.*, 1938, **68**, 353.
2. McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 347.
3. McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 373.

4. Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57**, 751.
5. Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1932, **56**, 223.
6. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **56**, 239.
7. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1934, **60**, 479.
8. Burton-Opitz, R., A text-book of physiology, Philadelphia, W. B. Saunders & Co., 1920.
9. Lohmann, A., in Ellenberger, W., and Scheunert, A., Lehrbuch der vergleichenden Physiologie der Haussäugetiere, Berlin, Paul Parey, 1910.
10. von Leerson, E. C., *Arch. Physiol.*, 1911, **142**, 377.