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In this second lecture, Starling's extraordinary insight is once again vividly displayed. Despite numerous embellishments the basic principles of lymph production (see Lecture I, Lymphology 17:83-88, 1984) and tissue fluid absorption (see below) are as sound today as when first proposed almost 90 years ago. (CLW)

The Arris and Gale Lectures on the Physiological Factors involved in the Causation of Dropsy

Lecture 2. The Absorption of Fluids from the Connective Tissue Spaces

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We have now to consider the second physiological factor which is involved in the production of dropsy. Two ways of absorption have been generally described—viz., absorption by the lymphatics and absorption by the blood vessels; and we find that at different periods in the history of the subject each of these has in turn been looked upon as the most important, or even exclusive, channel. There can be no doubt that absorption may take place by both these ways. Since the mechanism of absorption by the lymphatics is the more evident and familiar way we may consider this way first.

All parts and organs of the body possess a well-developed system of lymphatics. These channels are in direct communication with the tissue-spaces and can be easily injected from these spaces. They are lined by a layer of endothelial cells, which are continuous except at the very point of junction of the lymphatics with the tissue spaces. The larger trunks possess also an outer wall of connective tissue in which in some situations we find unstriated muscular fibers. All the lymphatic trunks, with the exception of those draining the upper extremity and right side of the head and neck with the adjacent parts of the thorax, converge to join the thoracic duct, which pours its contents into the blood in the angle between the left jugular and subclavian veins. All the larger lymphatics possess numerous valves so arranged that the lymph can only flow in them towards the thoracic duct. It is evident from these anatomical arrangements that any excess of fluid in the connective tissue spaces will make its way into the lymphatics and so finally attain the blood by way of the the thoracic duct. There is one point, however, which I think is not sufficiently appreciated, and that is the importance of muscular movements both for emptying the spaces into the lymphatics and for propelling the lymph onward through the lymphatics. If we have a manometer in the peripheral end of one of the lymphatics of the leg and inject fluid under pressure into the connective tissue spaces of the foot we find that the manometer in the lymphatic rises extremely slowly, so that the pressure in the spaces may be many times the pressure that obtains in the lymphatic. We have now only to flex the foot on the leg once or twice to immediately send up the pressure in the lym-

phatic to a height equal to or above that in the connective tissue spaces. Our knowledge of the importance of muscular movement for the normal lymph-flow we owe to the labours of Ludwig and his pupils. In all tendons and aponeuroses we find a double system of lymphatics—a deep system, consisting of vessels running parallel to the tendon-bundles and communicating by cross branches, and a super ficial network with polygonal meshes in the peritendinous connective tissue. Both networks are in direct communication and neither of them possess any valves. The superficial network drains into larger lymphatic trunks provided with valves. Each time that the underlying muscle contracts the deeper system is pressed upon and its contents emptied into the superficial system and so into the valved lymphatic trunks. When the muscle relaxes the fluid cannot get back past these valves, so that the two systems of lymphatics are emptied and ready to receive more fluid from the connective tissue spaces. A similar double system was described long ago by Ludwig and Schweigger-Seidel in the central tendon of the diaphragm, and these writers showed how beautiful injections might be made from the peritoneal cavity by injecting Berlin blue into this cavity and then carrying out artificial respiratory movements. As I have mentioned in my last lecture, it is impossible without such aid as muscular movements or passive flexion and extension of the limb to get any lymph-flow at all from a cannula placed in the lymphatics of the leg or arm. It will be evident to you therefore what an imperfect conception of the normal lymph-flow we obtain from a study of the flow in an animal anesthetized and secured on its back on the operating table, when during normal existence every breath we draw and every movement or twitch of the muscles helps to pump lymph from the extremities into the thoracic duct and so into the blood.

Much more difficulty exists with regard to the absorption of fluid from the connective tissue spaces by the blood vessels, and this question has been the subject of many investigations. Many years ago Magendie devised a number of ingenious experiments in order to show that poisons introduced into the serous cavities ad connective tissue spaces of the limbs were absorbed not by the lymphatics but by the blood vessels. These researches have been continued and extended of late years by Ascher in the case of the connective tissues of the lower limbs, by Tubby and myself in the case of the pleural and peritoneal cavities. We found, for example, that after injecting methylene blue or indigo carmine into the pleura the dye-stuff appeared in the urine within five minutes, whereas the lymph presented no trace of blue for another twenty minutes or even two hours. It is evident then that in this case the dye must have been taken up by the blood vessels and not by the lymphatics, and that this vascular absorption takes place with extreme rapidity. In a later series of experiments Leathes has shown that after introduction of various salt solutions into the serous cavities an interchange of constituents takes place directly between the blood and the injected fluid, so that the latter in a very short time becomes isotonic with the blood plasma. Now in this mode of absorption by the blood vessels the so-called absorption really consists in an interchange between blood and extra-vascular fluids—an interchange apparently dependent entirely upon processes of diffusion between these two fluids. So long as any difference in composition exists between intra-and extra-vascular fluids so long will diffusion-currents be set up tending to equalise this difference. The power of interchange between blood and tissue juices is probably of great importance in the normal metabolism of the tissues. Supposing, for example, that a tissue cell is storing up in itself a large amount of calcium or secreting it away from the tissue fluids into the lumen of a gland duct, it is evident that the percentage amount or partial tension of the calcium salts in the fluid surrounding the cell will be diminished. Diffusion currents will therefore be set up, and there will be a flow of calcium from the blood to the lymph until normal calcium tension is restored. We must conclude, therefore, that for the ordinary metabolic activities of the tissues no lymph-flow at all is necessary, the cell being able to obtain all its diffusible food stuffs from the blood by means of diffusion even through a stationary layer of lymph.

These experiments, however, have no direct bearing upon the absorption of dropsical fluids by the blood vessels. In the case of dropsical fluids the exudation resembles in all points blood plasma, the only difference between the two consisting in the fact that the dropsical fluid is rather poorer in proteids than the plasma. Here, as we have fluids almost identical on each side of the endothelial wall of the vessels, no conditions are present which would favor the setting up of diffusion currents. As a matter of fact, we may say that diffusion currents bring about an interchange of constituents between the two fluids, but can under no circumstances effect a total absorption of fluid. We have therefore to inquire whether, under any circumstances, an absorption of dropsical fluids or of isotonic salt solutions can be carried out by the blood vessels. We may arrange the experiments upon this point under three headings.

1. In the first set observations were made on the absorption of isotonic salt solutions and blood serum *from the pleural and peritoneal cavities. Orlow, working under Heidenhain's direction, found that such fluids were absorbed rapidly from the peritoneal cavities of living animals, while the lymph-flow from a cannula placed in the thoracic duct showed no (or only slight) increase in no way comparable to the amount of fluid absorbed. He concluded, therefore, that the absorption was affected by the blood vessels, and was dependent on the vital activity of the cells lining the serous cavities or of the endothelial cells of the capillaries. Leathes and Hamburger confirmed these results, but showed that they could not depend on any vital activity of the endothelial cells, since absorption took place with equal rapidity even when poisonous solutions of sodium fluoride were made use of. The great objection to these experiments is that they do not prove conclusively absorption by the blood vessels. It is still possible that the fluids had been taken up by the sub-serous lymphatic network and had not reached the thoracic duct during the experiment. This is an objection raised by Cohnstein, who concludes from very similar experiments that these fluids are carried away solely by the lymphatics. It might be thought that this question could be easily decided by observing whether fluids were still taken up from the serous cavities after ligature of both thoracic ducts. I have made a number of experiments of this description, but have failed to get decisive results. It is true that after ligature of both thoracic ducts as well as of the right innominate vein isotonic salt solutions were taken up fairly quickly from the serous cavities. In none of these cases, however, could I be certain that the lymph was absolutely shut off from the blood. As a rule I injected on three succeeding days several hundred c.c. salt solution into the peritoneal cavity, the last injection containing carmine granules in suspension. On killing the dog two days after the last injection the peritoneal cavity was generally found to be empty, and carmine granules could be traced along the glands of the anterior mediastinum, showing that, in spite of the ligature of both thoracic ducts, there had been a passage of lymph upwards and through the chest. We must, therefore, look to other methods to decide this question.*

2. There are a whole series of experiments made by other observers which to my mind prove conclusively the power of the blood vessels to take up fluid from the tissue spaces. If an animal be bled several times it will be found that the blood obtained in the later bleedings is more watery than that obtained at the beginning of the experiment. Now this diminution of total solids in the blood seems to be due chiefly to a dilution of the serum; the serum contains less solids than before and is increased in volume relatively to the blood corpuscles. I may here quote some observations which show this point.

Dog, 11.4 kilos. Solids of serum = 7.72 per cent. Dog then bled to 220 c.cm. Thirty minutes later solids of serum = 7.14 per cent.

In another experiment the solids of the serum were at first 6.98 per cent.; after bleeding to 200 c.cm. = 6.57 per cent.; after further bleeding to 100 c.cm. = 6.37 per cent.

In a smaller dog (6.5 kilos.) withdrawal of 150 c.cm. blood reduced the solids of the serum from 7.77 per cent. to 6.47 per cent.

It must be noticed that this attempt to regulate the amount of the circulating blood by bringing it up to its normal volume is carried out with great rapidity, so that even while we are bleeding an animal we find that the later portions of blood are more dilute than the earlier portions. That the fluid which is added to the blood in these cases is derived from the tissue spaces is shown by Barlow's experiments. Now this dilution of the blood takes place even when the thoracic duct is tied or when the lymph is conducted away by placing a cannula in the duct, so that it cannot be due, as was formerly thought, to an increased lymph-flow into the blood.

3. In order to be absolutely certain of the power of the blood vessels to take up isotonic solutions and dropsical fluids from the tissue spaces I have carried out a series of experiments in which I led defibrinated blood through the blood vessels of amputated limbs. In each case I had a double set of transfusion apparatus and sent one-half of the blood many times through a limb which had been rendered dropsical by the injection of isotonic salt solution, while at the same time fluid was flowing at the same pressure through the other limb, which was not dropsied, and thus served as a control. In each case the blood was analysed and its haemoglobin estimated before the experiment and from both limbs after the experiment. It was invariably found that, whereas the blood which had passed from twelve to twenty-five times through the sound limb had become rather more concentrated, the blood which had passed through the oedematous limb had taken up fluid from this limb. I may here quote one of these experiments as an example:

	Total Solids	Percentage of HbO_2
1. Blood before experiment	21.2%	100
2. After twenty passages through normal leg	21.4%	103
3. After twenty passages through oedematous leg	20.5%	95.5

We must conclude, then, that isotonic salt solutions and dropsical fluids may be taken up by the blood circulating through the capillaries and that this process may occur fairly rapidly. In the last lecture we saw how any excess of intracapillary pressure, such as accompanies plethora, causes an increased transudation form the capillaries, so that volume of circulating fluid is diminished. Now we see that, on any diminution of capillary pressure taking place, as after bleeding, the fluid in the tissue spaces goes back into the vessels to make up for the volume of circulating fluid lost. This wonderful balance between capillary pressure and lymph production or absorption is, I think, well illustrated by Lazarus Barlow's observations. While not agreeing with my views on lymph-production, this observer has shown that the slight plethora produced by wrapping up the limb in Esmarch's bandage causes an appreciable increase in the transudation in other parts of the body, so that the specific gravity of the tissues of the upper limb, for instance, falls, while the specific gravity of the blood rises. The reverse is the case when circulation is restored to a limb which has been kept anaemic for an hour or two. Here there is considerable hyperaemia of the affected limb produced, and corresponding anaemia of other parts of the body. We find then that absorption as well as transudation through the capillary wall is determined by the intracapillary pressure. When the pressure rises transudation is increased, when the pressure falls absorption is increased. We have seen that the dependence of transudation on capillary pressure is susceptible of a fairly simple mechanical explanation (filtration hypothesis). We have now to discuss the mechanism of the absorption process.

Is absorption effected by the active intervention of the endothelial cells or are there physical factors at work which will serve to explain it? An explanation of absorption which will strike anyone who investigates this problem is the possibility that absorption may take place in the same manner as lymph production—i.e., by a process analogous to filtration. From the works of Ludwig and Noll we know that the pressure in the lymphatics is extremely low; but the experiments I mentioned earlier in this lecture show that there may be a considerable pressure in the tissue-spaces without communicating itself to the fluid-filling lymphatics. Landerer, who has written a whole book on the subject of tissue tension, places the pressure in these spaces at a high level, from one half to three-quarters of the capillary pressure. It is evident that if this were the case and the capillary pressure suddenly sank 50 per cent. the pressure in the extravascular spaces would be higher than that in the capillaries and a backward filtration of lymph might occur. A series of mechanical experiments by Klemensiewicz would seem at first sight, however, to show that such a backward filtration is impossible. Klemensiewicz points out that if fluid be passing are a given pressure through a permeable tube contained within a rigid tube transudation will occur until the pressure of the transuded fluid is equal to that of the fluid flowing through. At a certain point in the experiment the pressure of the transuded fluid will exceed the pressure at the outflow end of the tube. The tube will collapse and the flow through it be stopped. He imagines that the same sequence of events occurs in the living body in the presence of a considerable transudation. Arteries, capillaries, and veins are bathed in the transuded fluid. The fluid transuded from the capillaries will, if a free outflow for it be absent, after a time attain a pressure near that ruling in the capillaries and higher than that of the venous pressure. The veins will therefore collapse, venous obstruction will be produced, and the capillary pressure and transudation will be higher than ever, so that we have a vicious circle of events tending continually to increase the oedema of that part. Now, Klemensiewicz's objections are true only under one condition—i.e., that the venous tubes should run freely through the lymphatic spaces of the tissues. If, however, we consider a system in which the inner tube is connected at various points in its circumference to the outer tube by strands of inextensible fibers, it is apparent that a rise of pressure in the space surrounding the inner tube will only serve to extend this tube still further. No collapse will take place, but a back filtration will be possible. Now if we cut sections of injected connective tissues we find that the capillaries are bound to surrounding parts by radiating fibers which might possibly prevent their collapse under high extravascular pressure. In the larger veins, on the other hand, the arrangement of the fibers of the adventitia is circular and not radial, so that a high extra-vascular pressure would apparently cause the collapse of the veins. From these anatomical facts one would conclude that a backward filtration is possible provided that the extravascular pressure be raised only in the region of the capillaries. If, however, the pressure be freely propagated through the tissues so as to affect the larger veins draining them we shall have collapse of the veins and increased oedema. Here, as in so many other cases, we cannot get a decisive answer to our physiological questions by purely anatomical investigation, but must have recourse to physiological experiment.

The question that we have immediately to decide is whether an increased tissue tension augments or leaves unaltered the flow of blood through the tissues or whether it causes venous collapse and so diminishes the flow. In the former case *a back filtration would be possible and in the latter case impossible. I have investigated this point in various regions of the body, the connective tissues of the leg, the tongue as a type of muscular tissue, and the submaxillary gland as a type of glandular tissue. In all these cases I have found that a rise of tissue tension above the pressure in the veins causes collapse of these veins, a rise in capillary pressure, and a diminished flow of blood through the part. In these regions of the body, therefore, absorption of lymph by a backward filtration is impossible.*

Hamburger, finding that serum and isotonic fluids are absorbed from the peritoneal cavities that had been dead some hours, concluded that the life of the endothelial cells can have nothing to do with the process and ascribes the absorption to processes of capillary and molecular imbibition, so that the absorption of fluids would be analogous to the taking up of fluids and gases by animal charcoal. Though these factors probably cooperate to a certain extent in the distribution of the fluid through the tissues surrounding the serous cavities, it is evident that they would be much more pronounced in dying and disintegrating tissues, and could with difficulty explain the taking up of fluids by the blood vessels. They would certainly not explain the wonderful balance which exists between the intracapillary pressure and the amount of fluid transuded from or absorbed by the blood vessels. What, then, is the explanation of this absorption? The explanation is, I believe, to be found in a property on which much stress was laid by the older physiologists and which they termed the high endosmotic equivalent of albumin. It must be remembered that the older physiologists used animal membranes in their experiments on osmotic interchanges. These membranes permit the passage of water and salts but hinder the passage of coagulable proteid. The application of semipermeable membranes to the measurement of osmotic pressures showed that the osmotic pressures of salts and other crystalloids are enormously higher than those of colloids such as albumin, and it has therefore been supposed that the osmotic pressure of the proteids in the serum being so insignificant must be of no account in physiological processes. The reverse is, however, the case. Whereas the enormous pressures of the salts and crystalloids in the various fluids of the body are of very little importance for most physiological functions, the comparatively insignificant osmotic pressure of the albumins is of great importance and for this reason. It has been shown that bodies in solution behave in most respects like gases. Now there can be no difference in pressure between two gases in a vessel which have no partition between them or only divided by a screen freely permeable to both gases. In the same way if we have two solutions of crystallised substances separated by a membrane which offers free passage to the water and the salts on either side there can be no enduring difference of the osmotic pressure on the two sides, especially if a free agitation of the fluids on both sides is kept up. The pressure on the two sides will be speedily equalised, then any flow of fluid from one side to the other will cease. Now the capillaries in the living body represent such a membrane. Leathes has shown that within 5 minutes after the injection of sugar or salt into the blood vessels the osmotic pressures of the blood and lymph have become equal. Supposing, however, that we have on one side of this membrane a substance to which the membrane is impermeable, this substance will exert an osmotic pressure and will attract water from the other side of the membrane with a force proportional to its osmotic pressure. This attraction of fluid must go on until all the fluid has passed through the membrane to the side where the indiffusible substance is.

Now the capillaries of the body are almost impermeable to proteids. In consequence of this impermeability the fluid which is transuded from the capillaries under pressure contains very little proteid. From what I have just said it follows that the proteid left in solution within the capillaries must exert a certain osmotic attraction on the salt solution outside the capillaries. It is easy to measure the value of this attractive force. If we place blood serum in a small thistle funnel over the open end of which is stretched a layer of peritoneal membrane soaked in gelatin, and immerse the inverted funnel into salt solution which is isotonic or even hypertonic as compared with the serum, within the next two days fluid will pass into the funnel and will rise in its stem to a considerable height. I have found that the osmotic pressure of the non-diffusable portions of blood-serum measured in this way amounts to 50 mm. Hg. Now you will see at once the importance of this fact. Although the osmotic pressure of albumin is so insignificant, it possesses an order of magnitude comparable to that of the capillary pressures, and whereas capillary pressure determines transudation the osmotic pressure of the proteids of the serum determines absorption. Moreover, the osmotic attraction of the serum for the extravascular fluid will be proportional to the force expended in the production of this extravascular fluid, so that at any given time there must be a balance between the hydrostatic pressure of the blood in the capillaries and the osmotic attraction of the blood for the surrounding fluids. With increased capillary pressure we shall have increased transudation until we get equilibrium established at a somewhat higher point, when there is a more dilute fluid in the tissue spaces, and *therefore a higher absorbing force to balance the increased capillary pressure. With diminished capillary pressure there will be an osmotic absorption of salt solution from the extravascular fluid until this becomes richer in proteids, and the difference between its osmotic pressure and that of the intravascular plasma is equal to the diminished capillary pressure.*

Here, then, we have the balance of forces necessary to explain the accurate regulation of the quantity of circulating blood, according to the conditions under which the animal may be placed, and it seems unnecessary to invoke the aid of vital activity to explain the process. Certain corollaries of this mode of explanation agree well with observed facts of experiment. Thus, the more impermeable the capillary the smaller will be the amount of proteid exuded with the lymph. A higher capillary pressure will therefore be needed in its production and there will be an equally high force tending to its reabsorption. A rise of capillary pressure will only increase the amount of lymph in the extravascular spaces to a certain extent, but will at the same time cause this lymph to be more dilute, so that there will be a corresponding rise in the force tending towards absorption. In consequence of this sequence of events considerable alteration of capillary pressure may be produced in impermeable capillaries such as those in the limbs, without causing any appreciable increase in the lymph-overflow from the limbs. On the other hand, where the capillaries are very permeable very little pressure will be required to cause a transudation, since no work is done in the concentration of a proteid solution, and we find, as a matter of fact, that capillaries where the pressure is lowest—i.e., in the liver—are also those which are the most permeable. Here, moreover, the absorbing force will be insignificant, since there is very little difference in the percentage of albumin between liver blood and liver lymph.

The osmotic difference between blood plasma and tissue fluid will not serve to explain the absorption of proteids by the blood vessels and would certainly not explain the absorption of serum from the serous cavities. It is difficult, however, if not impossible, to prove that serum or proteid is absorbed by the blood vessels. In some of my transfusion experiments I have rendered a limb oedematous by means of serum, and in these cases have obtained no evidence at all of absorption by the blood vessels. There is no doubt that serum may be absorbed form the pleural and peritoneal cavities, but the absorption of these fluids is very much slower than the absorption of salt solutions, and is in fact, so slow that the whole of it can in most cases be effected by the lymphatic channels. A slow absorption of serum from tissue spaces by means of the blood vessels is also physically possible. As the cells of the tissues feed on the proteids of the fluid the serum will tend to become weaker and weaker, so that the watery and saline constituents corresponding to the proteid used up can then be absorbed by the blood vessels in the way I have indicated.

The physical process which I have described above as causing the absorption of lymph by the blood vessels must be in action at all times in the body and must, therefore, be a predominant factor in the process of absorption. I have not been able to absolutely exclude the absorption of proteids by the blood vessels, but, in the absence of direct experimental evidence that such an absorption does occur, the physical factors I have described in this lecture suffice to explain the phenomena of absorption observed both under normal and under pathological conditions.