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CLASSICS IN LYMPHOLOGY

What makes a classic is both its timeliness and timelessness. By these criteria, Starling's epic work eminently qualifies. As can be gleaned from careful reading of his following "lecture" and other work, Starling not only recognized the forces regulating transcapillary fluid flux but fully anticipated issues surrounding macromolecular transport, regional variations in microvascular permeability, and "safety" factors forestalling edema (e.g. widened transcapillary oncotic gradient, accelerated lymph absorption and autoregulation of capillary pressure by reduction in arterial inflow during venous outflow block). A comprehensive up-to-date review of "our understanding of the wide variety of pathologic processes that share the common property of edema formation" has just been published (Edema: NC Staub and AE Taylor, Editors. Raven Press, 1984) embodying, embellishing, and advancing concepts first elucidated by Starling. (MHW)

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

Lecture 1. The Production of Lymph

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Delivered before the Royal College of Surgeons of England, 1896.

The term dropsy is used to denote the condition in which there is an abnormal accumulation of lymph in the connective tissue spaces, including the serous cavities of the body. Under normal circumstances these spaces contain lymph, but the amount present never exceeds a certain limit. Under no physiological conditions can we speak of the spaces as distended with fluid. Now, the lymph in these spaces is derived from the blood plasma circulating in the capillaries. From these in all states of activity the lymph is transuded, but as fast as it tends to accumulate in the spaces it is removed by an absorbent apparatus represented chiefly by the lymphatics. As I shall show in my second lecture, the lymphatics are aided in this work of absorption, under probably certain defined conditions, by the blood capillaries themselves. In health, therefore, the two processes of lymph-production and absorption are exactly proportional. Dropsy depends on a loss of balance between these two processes—on an excess of lymph production over lymph-absorption. A scientific investigation of the causation of dropsy will therefore involve, in the first place, an examination of the factors which determine the extent of these two processes and, so far as is possible, the manner in which these processes are carried out. In the second place, we must inquire how it is that, under the clinical conditions in which we know dropsy to occur, there is an upset of the balance of these two processes in favour of lymph production. My first lecture, then, will deal with the first of the two processes—i.e., Lymph-Production.

In studying the factors which regulate the production of lymph we must have criteria by which we may judge of the amount of lymph produced. To this end several methods are open to us. The most simple method, and one which was the earliest to be used, is the production of edema. As a rule, I shall explain later, we may look upon the production of edema as due to increased lymph-production. Far more delicate, however, than this method is the observation of the lymph-flow from a cannula placed in a *lymphatic trunk, which drains the part under investigation. Most delicate of all, but subject, perhaps, to more fallacies, is the ingenious method devised by Roy and used by Lazarus-Barlow, in which the specific gravity of the tissues is determined. The second of these methods is the one by which most work has been done and which I have used almost exclusively in my own investigations. It is evident that the only constant source of a lymph-flow must be the blood vessels, and our first object must be to see how the amount and composition of the lymph formed in any given part may be changed by experimental alterations of the pressure and chemical composition of the blood flowing through the capillaries of that part as well as by changes in the walls of the capillaries themselves. In dealing with the functions of any part of the body we can often obtain hints as to the manner of its working from a study of its structure, and I would like, therefore, in the first place to draw your attention to some points in the structure of blood capillaries. These have long been known to consist of tubes whose walls are formed of a simple layer of flattened, nucleated endothelial cells, which are united together by a small amount of cement substance, as it is called, the lines of junction between the cells staining deeply with nitrate of silver. A Russian observer, Kolossow, has added somewhat to this simple account. According to this observer each endothelial cell consist of two parts, a hyaline "ground-plate," which immediately borders the lumen of the capillary, and outside this a protoplasmic granular part in which is embedded the nucleus. The ground-plates of the adjoining cells come in intimate contact with one another, but are in no way continuous, so that there is a linear cleft between the adjacent portions of every two cells. The protoplasmic portions of the cells, on the other hand, are continuous with one another by means of processes. If the capillary be stretched in any way the ground-plates, which are inelastic, are separated from one another, so that the clefts between them are increased in size. Hence we see in nitrate of silver specimens made under these conditions that the lines of reduced silver are much broader than in normal capillaries. Outside the capillary one finds in most instances an ill-developed adventitia which is continuous with the surrounding connective tissues. The picture thus afforded of a capillary must suggest at once that it will not be, so to speak, water-tight, but will permit of filtration between the cells, and if this were the case we would have to look upon the lymph, which is separating from the plasma circulating through the capillaries, as a filtrate. The fact that the lymph from most parts of the body contains less proteid than the blood plasma is no argument against this hypothesis. If we filter serum through porous filter paper the filtrate will have the same composition as the original serum. If, however, we take a more closely-meshed filter, such as a porous clay cell or an animal membrane, we shall find that the filtrate is considerably poorer in proteid than the original serum, the big proteid molecule being apparently unable to go through the smaller pores of such a filter. Now this hypothesis, that the lymph is to be looked upon as a filtrate under pressure from the plasma, has been the guiding idea in the most important works on lymph-formation which have appeared during this century. Though we find it more or less distinctly in the works of the older writers—Hales, Hewson, and others—it is to Ludwig and his pupils that we owe its most systemic examination. According to this hypothesis, the amount of lymph produced in any given part must be proportional to the difference between the pressure in the capillaries and the pressure in the extra-vascular spaces. In most of Ludwig's earlier experiments on the subject this condition was found to hold good. On leading defibrinated blood through a limb the lymph-production in the limb was found proportional to the pressure at which the blood was led through it. In the testis ligature of the pampiniform plexus was found to increase largely the lymph production in this organ. In the arm and in the leg extensive ligature of the veins led to an increased flow of lymph. In all these cases, therefore, an increased flow of lymph was obtained by increasing the capillary pressure of the part. Ludwig found it more difficult to prove any constant alteration of lymph production incident on vaso-motor changes although Rogowicz, working later in Heidenhain's laboratory, found that vasodilatation did give a certain definite increase in lymph-production, and also showed clearly that the vasodilatation of the tongue produced by excitation of the lingual nerve was followed by an increased lymph-production in the tongue, which might at times amount to an actual unilateral edema of this organ.*

In dealing with the laws affecting lymph-production one is hampered by the fact that in the limbs of an animal at rest there is under normal conditions no lymph flow at all, so that when we wish to study the effects of our various procedures on the lymph-production in the limb we have artificially to bring about a lymph-flow by kneading and massaging the limb. Now this fact introduces at once an arbitrary element into the experiment, and Heidenhain suggested, therefore, that the best place to investigate the truth of the filtration hypothesis would be on the lymph-flow from the thoracic duct. This writer, therefore, carried out a long series of researches on *the various conditions in which the lymph-flow from the thoracic duct might be increased or diminished, and came to the conclusion that the results of his experiments were irreconcilable with the filtration doctrine and that we must assume that the cells forming the walls of the capillaries take an active part in lymph-formation—i.e., that lymph must be looked upon as a secretion rather than as a transudation. A renewed examination of Heidenhain's experiments, combined, however, with a more thorough investigation of their conditions, has shown me that, so far from overthrowing the filtration hypothesis, they furnish the strongest arguments which have yet been adduced in its favour.*

In dealing with the lymph-flow from the thoracic duct it is essential to know from what parts of the body this lymph is derived, especially since, as is well known, the lymphatics from all parts of the body, with the exception of the right upper extremity and right side of the neck, converge to pour their contents into this duct. In placing a cannula in the duct in order to collect and measure the lymph the ducts from the left side of the neck and left upper extremity are ligatured. From the hind limbs we know that in an animal at rest on the table there is no lymph-flow at all. Hence the sources of the lymph are confined to the trunk. We can, moreover, exclude the thorax and its contents, since ligature of the thoracic duct just above the diaphragm absolutely stops the lymph flow. Therefore, when dealing with the lymph-flow from the thoracic duct we deal only with the lymph coming from the abdominal viscera. As I shall show presently, the abdominal viscera, so far as their lymph is concerned, may be divided into two groups; (1) the viscera drained by the portal vein, and (2) the liver.

In testing the filtration hypothesis on the lymph-flow we have to investigate whether the flow is always proportional to the difference between the intra- and extra-capillary pressures. We may regard the extra capillary pressure as not varying to any large extent, so that we have to see what effect is produced on the lymph by variations in the intra-capillary pressure in the intestines and the liver. The simplest experiments on the subject are those in which some large vessel is obstructed. Speaking generally, we may say that obstruction of a large vein raises the pressure in the capillaries immediately behind it, whereas obstruction of an artery will diminish the pressure immediately in front of it. If, for instance, we ligature the portal vein the arterial pressure is very little affected, while the pressure in the vein behind the ligature rises enormously. In consequence of this there is a large rise of pressure in the capillaries of the intestines and spleen, so that the spleen swells and the intestines become black from venous congestion and hemorrhages are produced into their mucous membrane. The effect of this ligature on the lymph-flow from the thoracic duct is to increase it four or five times. The lymph also becomes bloody and its total solids are diminished. The diminution in solids is due solely to a diminution in proteins, the salts remaining the same as before, so that we have here an increased capillary pressure, causing an increased transudation of lymph containing a diminished percentage of proteid—a result which is also obtained when proteids are filtered with pressure through dead animal membranes. The presence of red blood corpuscles in the lymph is not a necessary consequence of a rise of pressure in the portal vein. If a less excessive rise of pressure be produced by ligaturing the vein, not at its entry into the liver, but just below the pancreatic duodenal vein, thus leaving a circuitous route for the blood to the liver through the anastomoses of this branch, an increased flow of lymph is produced containing less proteids than normal lymph, but which may be quite free from blood corpuscles.

A still more striking effect is produced by obstructing the vena cava just above the diaphragm. The lymph is increased from ten to twenty-fold, and it is found that the lymph obtained after the obstruction is free from red blood corpuscles and is more concentrated than normal lymph. What is the cause of this increased lymph-flow and why is it more concentrated? To answer these questions we must find out, firstly, the source of the lymph, and, secondly, the condition of the capillary pressure in the organ or organs from which the lymph is derived. We can determine the source of the lymph by a process of exclusion. Ligation of the kidney vessels and lymphatics has no effect on the usual consequences of obstructing the inferior vena cava. On the other hand, if we ligature the lymphatics in the portal fissure which carry off the liver lymph we find that a subsequent obstruction has no effect on the lymph-flow, or, indeed, may slightly diminish it. We must conclude then that the increased flow of lymph is more concentrated than intestinal lymph. In order to answer the second question as to this increased production of lymph in the liver we must investigate the changes in the circulation produced by the obstruction. On obstructing the inferior vena cava and recording the blood pressure in the chief vessels of the abdomen we notice that the pressure in the aorta drops almost at once to a third of its previous height, whereas there is a very considerable rise of pressure both in the portal vein and inferior vena cava. It is probable that the effect of the rise of portal pressure on the intestinal capillaries is more than counterbalanced by *the severe fall in arterial pressure, so that there is a fall of pressure in the intestinal capillaries. This conclusion is borne out by the fact that if the abdomen be opened the obstruction of the inferior vena cava is seen to be at once followed by a blanching of the intestines; on the other hand, the effect of the simultaneous rise of pressure in the portal vein and vena cava must be to raise the pressure in the capillaries of the liver three or four times the normal amount. We have, then, as the results of this experiment, no rise of pressure in the portal area and no increase of lymph-flow from the portal area, a large rise of pressure in the hepatic capillaries, and a very large increase of lymph-flow from the liver.*

The only other experiment of this nature which I need describe is one in which the thoracic aorta is obstructed. The results of this obstruction on the lymph-flow are somewhat variable. In most cases the lymph is diminished to one-half or one-third its previous amount; in a few cases the lymph is unaltered in quantity or even slightly increased. In all experiments the amount of proteids in the lymph is increased. Now if we investigate the state of the circulation under these conditions we find that obstruction of the thoracic aorta causes an enormous fall of pressure in the aorta below the obstruction and a corresponding fall in the portal vein, whereas the pressure in the inferior vena cava is unaltered or in some cases even slightly increased. We must conclude, therefore, that in the intestinal capillaries the pressure has fallen considerably below its normal limits, while in the hepatic capillaries the pressure is very little altered or may even be somewhat increased. Hence the only region of the body below the point of obstruction where the capillary pressure is not much diminished is the liver. Now we find that the liver is also the sole source of the lymph obtained under these circumstances. If the hepatic lymphatics be ligatured and the thoracic aorta be then obstructed, the flow of lymph from the thoracic duct is absolutely stopped. In those cases, therefore, the lymph production in the organs of the abdomen is found to be absolutely proportional to the changes of the capillary pressures in these organs. In another set of experiments we find that a marked increase in the lymph-flow is produced by a general rise of capillary pressure in all of the organs of the abdomen. Such a general rise of capillary pressure may be produced by the injection of large quantities of normal saline fluid into the circulation, giving rise to a condition of hydraemic plethora. Under such circumstances the lymph may be increased from 50 to 100 times in amount, and may in some cases run from the cannula in the duct in a steady stream. Now, in hydraemic plethora there are two changes in the circulation which might possibly be responsible for the increased production of lymph; firstly, the change in the composition of the blood, and, secondly, the increased pressure in the capillaries of the abdominal viscera. We can decide which of these two factors is responsible for the increased lymph-flow by a very simple experiment. Previously to injection 300 c.c. of normal saline we bleed the dog 300 c.c., so that after the injection the total amount of circulating fluid is the same as at the beginning of the experiment. In this way we entirely avoid any rise of capillary pressure, while we have diluted the blood to an even greater extent than in the experiments in which hydraemic plethora was produced. The effect of such a simple hydraemia is to increase the lymph-flow from 3 c.c. in 10 minutes to 4 or 6 c.c. in 10 minutes, whereas if hydraemic plethora were produced the lymph would be increased from 3 c.c. to 30, 50, or 100 c.c. in 10 minutes. It is evident, therefore, that in the production of this increased lymph-flow the all important factor is the rise of capillary pressure.

Exactly the same interpretation holds good for the action of a certain class of bodies which were grouped together by Heidenhain in the second class of lymphagogues. These include bodies such as salt, sugar, potassium, iodide, etc. The injection of a strong solution of dextrose (30 gms. in 30 c.c. water) into the veins of an animal causes a considerable increase in the lymph-flow from the thoracic duct. The lymph at the same time becomes more watery than at the commencement of the experiment. Heidenhain ascribes this effect to a specific excitation of the secretory activities of the endothelial cells. The effect, however, can be explained in a much more simple fashion. All these solutions have an osmotic pressure which is considerably higher than that of normal blood plasma. A solution of dextrose that should be isotonic with the blood plasma would contain from 5 to 6 percent of this body. When we inject a solution containing from 50 to 75 percent of dextrose it will attract fluid from the tissues until its percentage is reduced to 5 or 6 percent, that is to say, 45 c.c. of fluid containing 30 gms. of dextrose will attract water from the tissues until its total volume is increased to 500 c.c. Of course, this estimate is merely a rough approximation at the truth, since before the sugar has had time to attract all this fluid a considerable amount of it will already have left the vessel by diffusion. As a matter of fact, however, we find that injection of a strong solution of dextrose is followed in a few minutes by a considerable dilution of the blood, caused by an increase in its volume. In some experiments of von Brasol the volume of the circulating blood was thus increased to twice or three *times its previous amount; and these observations have been fully confirmed in a series of careful experiments made by J. B. Leathes. As we should expect, this increase in the volume of the circulating blood is attended by a large rise of capillary pressures in the abdominal viscera and we have here again to decide whether it is the rise of capillary pressure or the change in the chemical composition of the blood that determines the increased lymph-flow. This question can be solved by using the same method that we adopted when dealing with the production of the increased lymph-flow in hydraemic plethora. We can entirely obviate the rise of capillary pressure if we bleed first to 300 c.c. and then inject a concentrated solution containing 18 gms. of dextrose. In this case the fluid that is dragged by the sugar from the tissues into the blood vessels only just suffices to make up for the previous loss of blood. No hydraemic plethora is produced; there is no rise of capillary pressure, and there is no increase in lymph-flow, although an abnormally large amount of dextrose is present in the circulation. We must conclude, therefore, that to increase flow of lymph caused by injection of the second class of lymphagogues is entirely due to the rise of capillary pressure thereby induced, and is in no way conditioned by a stimulation of the secretory activities of the endothelial cells. There is one point in the effects of the injection of these bodies which has been looked upon as a strong argument for the secretory hypothesis, and which I must, therefore, mention shortly here. If we analyze the lymph and the blood at different periods after the injection we find that the amount of sugar in the blood steadily diminishes while the sugar in the lymph first rises to a maximum and then diminishes parallel with that in the blood plasma. At a given period after the injection it is found that the lymph contains more sugar than does the blood plasma, and this fact was held to point to an undoubted secretory activity of the endothelial cells in the production of lymph. This conclusion, however, is by no means justified. The lymph flowing at any given moment from the thoracic duct does not represent the transudation from the blood at that moment, but is derived from the lymph that has been formed some time previously. If we had a solution of sugar gradually diminishing strength flowing into a lymphatic trunk of the leg it is evident that this fluid would mix with the lymph in the other lymphatics through which it flowed on its way to the thoracic duct. Later, the solution of sugar would have displaced practically all the lymph from these channels and would flow through the thoracic duct almost undiluted. It would take, however, some considerable time to flow from the leg to the thoracic duct so that the outflow from the duct would represent not the fluid which was being injected into the leg at that moment, but the stronger solution which had been flowing in some time previously. If one compared, therefore, the percentage of sugar in the fluid flowing from the duct and in the fluid flowing into the leg lymphatic at different times after the beginning of the injection we should obtain a curve exactly similar to those obtained by Heidenhain after the injection of sugar into the circulation, and looked upon by him as undeniable evidence of secretory activity.*

The dependence of lymph-formation on capillary pressure is not, however, the only important relationship brought to light by these experiments. The amount and composition of the transudation through a membrane depend not only on the pressure at which the transudation is effected but also on the nature of the membrane. According to the permeability to the membrane, so the amount and composition in proteids of the transuding fluid will vary. You will doubtless already have noticed that after obstruction of the inferior vena cava the pressure in the intestinal capillaries, although it probably sinks below its normal height, is yet as high as that in the hepatic capillaries. Nevertheless, we get a very small amount of transudation through the intestinal capillaries and a very large amount through the hepatic capillaries. It is evident, then, that the permeability of the liver capillaries must be very much more marked than that of the intestinal capillaries. In the same way we may compare the permeability of the intestinal capillaries with those of the limb capillaries. Normally from the limb there is no flow of lymph at all, whereas a probably equal pressure in the intestinal capillaries suffices to give rise to a steady flow of lymph. If we ligature all the veins of the leg a lymph-flow may be set up. but such a flow is incomparable smaller than that produced on ligature for the portal vein. We can, therefore, arrange the capillaries of the body in a descending order of permeability, the liver capillaries being the most permeable and the limb capillaries the least permeable. I have already mentioned how, on filtering solutions of proteids through various membranes, the percentage of proteids in the filtrate increases with the permeability of the membrane. As we have seen, exactly the same thing holds good from the capillaries in the body. The lymph in the limbs, the filtrate through the impermeable limb capillaries contains only from 2 to 3 % proteids. That from the intestines contains from 4 to 6 percent proteids, while that from the permeable capillaries of the liver contains from 6 to 8 percent proteids—in fact, almost as much as the blood-plasma itself. It is conceivable *that we might alter the amount of lymph produced in any organ by changing, not the intracapillary pressure, but the filtering membrane—i.e., the endothelial wall of the capillaries. Such a change can be brought about in the body by various means. A whole group of bodies has been described by Heidenhain as his first class of lymphagogues. These substances, which are mostly of the nature of albumoses, can be extracted from various of the lower animals and include leech extract, mussel extract, crayfish extract, and commercial peptone. On injecting a small amount of any of these extracts into the blood vessels of an animal the lymph from the thoracic duct is much increased in quantity and becomes more concentrated. Now all these bodies are poisons; they alter the blood, diminishing its coagulability, and when given in sufficiently large doses cause a great fall of blood pressure in consequence of paralysis of the heart and vessels. I have shown that the changes in the circulation produced by these bodies are insufficient to account for the increased lymph-flow, but that the increased flow is due to an alteration of the capillary walls in the abdominal organs, especially in the liver. The hepatic capillaries become even more permeable than before, so that a pressure within them which is little above normal is sufficient to cause a great increase of transudation through them.*

Another substance which seems to act directly on the capillary wall is curare. This body, however, differs from the class of lymphogogues just mentioned in the fact that its chief action is on the vessels of the limbs. The effect of curare in increasing the lymph-production in the limbs was noticed long ago by Paschutin working in Ludwig's laboratory. Its direct action on the endothelial wall of the capillaries can be easily demonstrated in the living frog's web. It may be seen that after the injection of curare the capillary walls become apparently more sticky, so that the capillaries become filled with a number of leucocytes adhering to their walls. A still more potent method of altering the permeability of the limb capillaries is to plunge the limb into water at 56°C. for some minutes. If a cannula has been previously placed in one of the main lymphatics of the leg it will be noticed that, in a very short time after this scalding, lymph begins to drip spontaneously from the cannula. The lymph which is thus produced is much richer in proteids than is lymph from a normal leg. The amount of lymph flowing from the leg can now be varied within wide limits by altering the pressure in the capillaries either by ligature of the vein or artery, injection of salt solution, or production of vaso-motor paralysis. By this scalding, in fact, we may reduce the limb capillaries to the condition of liver capillaries.

In conclusion, from this study of the conditions of lymph-production in the various parts of the body we must conclude that the endothelial cells of the vessels take no active part in the production, their vital activities being confined to the maintenance of their integrity as a filtering membrane with properties differing according to the part of the body in which they happen to be situated. The amount and composition of the lymph transuded in any part are determined solely by two factors: (1) the permeability of the vessel wall and (2) the intracapillary blood pressure. The more permeable the capillary the greater is the amount of lymph transuded under any given pressure, the greater is its concentration in proteids, and the more easily is the amount of lymph altered by slight changes of pressure.

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