

# THE HISTOLOGY OF BLOOD AND LYMPHATIC VESSELS DURING THE PASSAGE OF FOREIGN FLUIDS THROUGH THEIR WALLS

## II. STUDIES ON ABSORPTION FROM SEROUS CAVITIES

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It is at once evident to any one who studies the enormous literature which has been published during the last century, on the absorption into the body of foreign material from the serous cavities, that the problems presented to the present day investigators in this field may be roughly grouped under four heads, as follows: 1) the localization of absorbing surfaces; 2), the definite establishment of the channels of removal; 3) the determination of the forces concerned in the passage of matter through the walls of the serous cavities, and its entrance into and exit from the vessels which distribute it about the body for storage and digestion, or for destruction and excretion; and 4) the location and identification of the organs concerned in the storage or destruction of substances or fluids absorbed.

Up to the present time not even the questions which are included under the first and fourth of these heads can be said to have been sufficiently answered; the second has been settled definitely only for a single area and with a limited number of substances, and about the third practically nothing is known.

Although a vast amount of work has been done on the localization of absorbing surfaces and their related organs of storage and excretion, until quite recently but little was known regarding them; and this is so in spite of their immense clinical importance in connection with the postural treatment of the various serositisides.

It is owing to the work of MacCallum (1), who made a careful and productive study of the removal of foreign granules from

the peritoneal cavity through the lymphatic lacunae of the diaphragm, that practically all who have made a study of serous absorption accept it as a fact that a great deal of any solution or foreign body introduced into the peritoneal cavity passes through the peritoneal surface of the diaphragm, and enters the system of the experimental animal or the patient via its vessels.

The position of other foci of drainage has remained unknown, and the tendency to deny or ignore their existence has been and is very strong.

A few observers have suggested the broad surface of the greater omentum as a possible agent in the removal of foreign fluid from the peritoneum, but their assertions have rested upon probability rather than definite proof.

Rubin (2), who attacked the problem from an experimental basis, showed, however, that less fluid was absorbed from the peritoneal cavities of animals whose omenta he had amputated, than from the peritoneal cavities of normal controls; and Crouse (3) after careful study concluded that the omentum is an important factor in the mechanism of peritoneal drainage, and hypothecates a protective lymphatic drainage to account for the phenomena which he has observed. The authors (4) have been able to show experimentally that beyond doubt the omentum is a very efficient agent in the drainage of the peritoneal cavity. By drawing the omentum out of the animal's body through a mid-line incision, and keeping it immersed in a fluid medium under physiological conditions, it was possible not only to isolate the organ and to prevent the experimental fluid from reaching other surfaces, but also to eliminate any influence on absorption which might be exerted by the increased abdominal tension which follows the intra-peritoneal injection of large amounts of the fluid. In spite of conditions which might be supposed to make for secretion rather than absorption, we found that a large amount of the fluid in which these omenta were immersed passed into the omental vessels, and reaching the general circulation, was carried at once by the blood stream to the organs of excretion, from which the test fluid could then be recovered.

As to the second question, the establishment of the vascular system concerned in the drainage, for example, of the peritoneum, Meltzer (5), Muscatello (6), and others, held that drainage is accomplished through the lymphatics, while Heidenhain (7), Cohnstein (8), Dandy and Rowntree (9), and others, have shown that much of the fluid absorbed from the peritoneal cavity leaves it through the blood vessels. We have never been able to demonstrate the presence of lymphatics in the omental tissue of the adult cat, and Ranvier (10) claimed that while there are lymphatics in abundance in the omenta of young kittens many of them are obliterated by degenerative changes at the age of three months. If lymphatics exist in the cat's omentum they must necessarily drain in the same direction as those of the gastric system; that is, an omental lymphatic stream, if such a thing exists, must eventually become tributary to the lymph content of the thoracic duct.

In the experiments mentioned above with the omentum of the cat, the influence of lymphatic vessels was entirely eliminated in many of our experiments by the ligation of the duct. Hence we were able to prove not only that the omentum furnishes a surface where absorption takes place, but, by varying the fluid in which the omenta were immersed, we have shown that the removal of molecular solutions and colloidal solutions and of fine particulate matter in true suspensions may be accomplished through the blood vascular system to a large extent; though we do not by any means deny the probability of drainage through the lymphatic channels in the localities where these vessels exist. But when any attempt is made to ascertain, through the medium of existing literature, the forces concerned in the absorption of foreign matter from serous surfaces one enters at once into a region of guess and hazard, where only a few isolated facts exist as a guide to certain knowledge. We have only just ceased to argue for and against the presence of preformed 'stomata' and 'stigmata,' and to indulge in surmises as to their physiological significance. Students of the physiology of absorption are still discussing whether absorbed material passes through or between the lining endothelium of blood and lymphatic vessels and the

mesothelial cells of serous cavities. In our own experiments we found that a great deal of fluid may enter the blood stream when the influence of intra-abdominal pressure is removed; and, since our fluids were isotonic with the blood serum of the experimental animal, osmosis as it is generally understood, could have had only a negligible amount of influence upon the phenomena observed. Indeed if osmotic pressure had any influence at all, it would seem that it would have been exerted against rather than for the passage of the experimental fluid into the blood vessels, since even the small amount of fluid lost from the solution by evaporation from exposure to the air, must have changed an originally isotonic to a slightly hypertonic fluid, to which one might expect water to pass from the serum through the vascular wall. If such a passage occurred it in no way interfered with the imbibition of the experimental solution. We know very little about the part played by fluid pressure, the movement of the blood and lymph in their respective vessels, or the influence on serous absorption of the movement of contractile somatic organs, like the diaphragm, or the contraction of the musculature of the vessels themselves. We cannot say what chemical changes accompany or influence the transport of material from cavity to vessel; or whether the cytoplasm of the cells of the serous cavities, or of the blood and lymphatic vessels play any part in the transmission of matter through the vascular or serous walls. And does a disturbed balance of intra- and extra-cellular equilibrium militate for or against absorption? We do not know.

It will readily be seen that the examination of histological preparations made from the omentum during active drainage, may be of great value in strengthening the positive evidence for absorption through the blood vessels, and in aiding us to understand the mechanism of the removal of foreign matter through the vascular wall.

With this end in view, sections have been made and studied of omenta which, up to the time of fixation, had been exposed to and were absorbing all sorts of material from true solutions to mechanical suspensions. A report of the findings in this material is the purpose of the present paper.

By far the most valuable preparations were yielded by omental tissue which had been absorbing an isotonic solution of potassium ferrocyanide and iron ammonium citrate, and which was fixed immediately upon removal from that fluid in hydrochloric acid formalin with a resulting precipitation of prussian blue—the method used by Weed (11) to study the drainage of the cerebro spinal fluid.

An omentum so treated appears in gross to be stained a uniform pale blue except for the fat, and is patterned by an irregular network of an intense dark blue color. It is only necessary to examine the spread preparations with a binocular microscope to be convinced that the network is made up of the omental blood vessels whose lumina are filled with precipitated prussian blue; the picture is strikingly suggestive of a complete blood vascular injection of the omentum with a somewhat dilute prussian blue gelatine mass, and, in fact, we are dealing with much the same thing, since the coagulation of the colloidal proteids during fixation of the blood serum causes the same comminution of the nascent dye stuff which follows its precipitation in peptizing gelatin. In contrast to the general tissue which fills the meshes of the vascular net and which is very pale blue, or uncolored, a wide deeply stained zone of thickly precipitated dye surrounds each vessel.

The capillaries are all filled with prussian blue, even those supplying the perivascular fat being crowded with the dye and the capillary knots or glomeruli which form the support of many of the taiches laiteuse are completely injected. Here and there a capillary may be seen empty or nearly so, perhaps because contraction of its walls during fixation forced the absorbed dye from its lumen.

Of the larger vessels all have a greater or less amount of dye precipitate within the lumen. All are completely filled, but in some the blue color is perceptibly paler than in others. In general the arteries show much less absorption than the corresponding vein, but the depth of the color may not be the same throughout the length of a given vessel. There are often light and dark blue areas present. These preparations show that ac-

tive absorption is going on through the walls of the arteries as well as through the veins, even arteries with thick muscular walls taking part in the general process as will be shown below.

The larger vessels are paler than the smaller, probably because less fluid is taken in through their walls. In other words as the vascular size increases there is a gradually decreasing concentration of the intra-vascular dye solution, the significance of which will be discussed below.

From the point where the vessels begin to be surrounded by a perivascular sheath of fat the pallor of the precipitated dye in the vascular lumen markedly increases, evidently because the advent of the perivascular fat is accompanied by an increased thickness of the vascular wall and a diminished absorption. That the fat itself can have no effect in the decrease is shown by the intense color of the injection mass in the capillaries supplying the fat; and examination of sections shows that the ferrocyanide solution penetrates easily between the fat cells themselves.

Sections of the same material confirm the evidence of total spread preparations. The capillaries, even those imbedded in and supplying the fat, are distended with the blue color, and the veins are full of blue precipitate of varying depth of color. It is however not so easy to see the blue color in the larger arteries in sections.

It is possible even in thin sections to distinguish the deep blue perivascular area described above, and to trace its existence to precipitated dye in the intrafibrillar tissue spaces. Individual fat cells are outlined by dye precipitated from the fluid which has worked its way between the cells that it has never penetrated. Coarse precipitates of dye may be seen along the surface of the omentum and adherent to the surfaces of the elastic fibres. In some places the tissue is diffusely stained, and throughout the omentum, cells, probably of the clasmatocyte type are found, like those described by Weed in the meninges, whose cytoplasm is filled with fine granules of prussian blue, their nuclei however remaining uncolored. This intracellular precipitate is the result of imbibition of fluid by the

connective tissue cells, an adsorption phenomenon of the same nature as the drinking in of solutions of high molecular vital dye stuffs which is responsible for the diffuse cytoplasmic coloration seen early in a course of staining.

The blood vessels contain many leucocytes, mostly of the mononuclear type, embedded in the precipitated blue of the injection mass. This prussian blue precipitate in the blood vessels is not the coarse amorphous mass in which that color is usually seen under the microscope, but because the dye was thrown down in the presence of the colloidal serum proteids it is so finely divided as to appear homogeneous except when examined with the highest power immersion lenses with which its finely granular nature can be ascertained. It is in the same physical condition of finely divided suspension as the silver in silver gelatine mixtures of photographers, or the dye granules in injection masses made by precipitating colors in the presence of solidifying gelatin. The intracellular precipitates and those adherent to the surface of the omentum and its component fibres are much more coarsely granular.

The endothelial walls of both capillaries and larger vessels are stained a dark blue. The cytoplasm of the endothelial and mesothelial cells is entirely filled with a fine granular precipitate of the prussian blue, and in some places dye particles are found apparently between the cells, though the walls of the cells are in such close apposition that it is difficult to say with certainty that such is the cause. The cell nuclei are uncolored, and the cytoplasm of the serous mesothelium covering the omental surface is filled with the fine granules of dye which show the track of fluid which has passed through their bodies.

By the time that the dye bearing serum has reached the visceral blood vessels—liver, lung, etc.,—it has become so diluted with blood from non-absorbing parts of the body that it is not possible to follow the course of the chromogen through the body by the examination of sections. Large quantities are present in the kidney tubules, and the presence of the dye in the urine of the animal can easily be demonstrated.

The same conditions prevail, though they are much more difficult to demonstrate, in preparations made from omenta which have been immersed in strong solutions of trypan blue and col-largol and colloidal solutions of other metals.

In animals which have been injected intraperitoneally with the chromogen solution—or where certain isolated portions of the peritoneal surface (small intestine or bladder)—have been immersed in, or covered with cyanide-citrate solution, and fixed in hydrochloric acid formalin, the blood vessels and lymphatics directly beneath the peritoneal surface, are found on section to be filled with dye precipitate and to have the same appearance as the omental vessels. Moreover it was possible in the gross to trace the stained lymphatic vessels directly to the lymph nodes into which they drained, and to obtain definite macroscopic evidence of the presence of prussian blue in the lymph gland. This feature of peritoneal absorption will be taken up separately in a later communication.

It is evident then from these histological preparations that there is very active absorption of foreign fluids through the peritoneal blood vessels, not only those in the omentum, but also through those beneath the peritoneum over the gut and bladder. In all probability fluids may be removed from the peritoneal cavity through any area in which blood or lymphatic vessels lie just beneath the peritoneal surface. Furthermore absorption of fluid obtains not only through capillaries, but through vessels of quite large caliber, and through arteries as well as veins, though not to as great an extent probably, because of the greater obstacles to fluid passage offered by the tissues which go to make up the thicker, denser arterial wall.

This is probably the reason for the pallor of the dye mass within the larger vessels, since it would seem reasonable to suppose that their thicker walls would hinder the passage of fluid and make it slower and of less amount. That fluids do pass through is shown by the fact that the wall and its lining endothelium contain granules of stain precipitated from the fluid during its passage into the vessels. That surrounding tissues have no significance in preventing fluid from coming in



contact with these large vessels is shown, as we have pointed out above, by the ease with which it penetrated between the cells in the perivascular fat and filled the capillaries by which the fat is supplied. There is of course the possibility of dilution of the chromogen fluid in the larger vessels as a result of their receiving blood from vessels through which absorption was not going on, but this is unlikely, since the vessels themselves and their entire tributary area were immersed in the test fluid.

The significance of the dark stained areas about the blood vessels is not quite clear. Apparently the solutions are drawn forcibly from the general tissue towards the blood vessels faster than they can be forced through the blood vessel wall, and then, removal being delayed (perhaps by the condensation of the connective tissue in the vascular margin) they are concentrated there. What the forces are which are exerted on the fluid, and what part is played by the movements of the omentum as a whole, the contraction of the blood vessels and the movement of the blood within them, it is impossible yet to say.

The material demonstrates also that while some fluid may pass between the lining cells of vessels on its way to their lumen, by far the larger part goes through the cytoplasm of the cells themselves. The sections are also of interest in that they show how little, if at all, the omentum was damaged during the operative procedure which preceded its immersion in the test fluid. Nowhere is there any sign of exudation or haemorrhage; there is no cellular death, as may be seen by the uncolored nuclei of the various cells; and, moreover, the mesothelial cells of the serous surfaces show no sign of disturbance or desquamation.

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