SOME OBSERVATIONS OF THE THORACIC DUCT LYMPH AFTER INJECTION OF OIL OF TURPENTINE INTO THE PERITONEAL CAVITY OF THE DOG.*

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The physical relation existing between serous cavities and the vessels of the lymphatic circulation has been studied in the past by many investigators, and efforts have been made to trace the paths taken by foreign material, soluble and insoluble, in leaving the abdominal cavity.

In this study we desired, particularly, to know if leucocytes that have entered the serous cavity as the result of an inflammatory process are returned to the circulation, and, if so, by what route. Some notice was also taken of the disposition made of insoluble pigment granules introduced into the abdominal cavity. In these experiments healthy dogs were used.

In order to establish the process of inflammation in the serous cavity, five to ten cubic centimeters of oil of turpentine were injected under morphin anesthesia through the abdominal wall by means of an ordinary syringe. In several instances a red dye was conveyed along with the turpentine into the abdominal cavity. At different intervals after the injection, the animals were examined.

Under anesthesia (chloretone) the thoracic duct was isolated in the neck, and a cannula introduced. Total and differential counts of the cells in the thoracic duct lymph were made in the usual way. The abdominal cavity was opened under anesthesia, care being taken to prevent blood from mixing with the fluid collected in the peritoneal cavity. Counts and study of types of cell in the effusion were then made. The observations and conclusions based upon this set of experiments are briefly stated in this paper.

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The amount and nature of the fluid found varied greatly with different intervals. While the changes as determined in different dogs were not identical, yet there existed a general relationship between the factors involved.

The *first or earliest effect* noted was a marked congestion of the vessels of the serous coat, this being accompanied by an effusion. The effusion had a very pale yellowish color, was of a watery consistency, and contained very few cells. These cells were of the small mononuclear type. After four to six hours the fluid clotted in a test-tube, forming a very soft, jelly-like mass.

It was not possible to determine the absolute number of cells per cubic millimeter of this fluid because of the fact that as the animals lay for some time in a given position the cells settled, and no particular quantity truly represented the total. Nevertheless, from the counts made of the cells in this early fluid the number was probably not more than two to three hundred per cubic millimeter.

Four to six hours after the injection of the turpentine, the cover-glass smears showed very few cells. Practically without exception these were the small lymphocytes and showed no marked variation in size. Red blood cells were absent. The fluid present was about fifty cubic centimeters.

At the end of the *second day* the effusion had increased to two hundred cubic centimeters. The color was more nearly pink, due to the increased number of red blood cells. The density of the fluid was greater, and it clotted in one to two hours into a more compact mass. The calculated number of the white cells per cubic millimeter was about ten thousand.

These cells varied greatly in many respects. The majority were mononuclears. Most of these were small lymphocytes and some were very small indeed, but many were unusually large, being much larger than the cells ordinarily found in the lymph or blood. Of these very large mononuclear cells there were about a dozen to a cover-glass. In diameter these cells were approximately thirty to forty microns. They had a large, compact, blue-staining nucleus with a peripheral band of protoplasm stained bright pink. These were probably mesothelial cells.

All the cells showed the effects of soaking in the fluid. This was

indicated especially by their frayed and indefinite outlines. Many of the cells were phagocytes and contained red blood cells, pigment, and cell detritus. Red blood cells were very numerous.

At the end of the *third day* the effusion amounted to two to four hundred cubic centimeters. The density and the cellular content were greater than on the second day and within half an hour the fluid clotted into a rather firm mass. It was of a light creamy color and so stringy as to make it difficult to make thin cover-glass smears. The smears showed a relative increase in the number of polymorphonuclear cells. There were many mononuclear elements showing much disintegration, and a great amount of a stringy fibrinous substance. The number of red cells was much decreased.

On the *fourth day* the amount of the fluid was much less, of a thicker, more creamy consistency, and clotted into a firm mass within half an hour. There was now a definite preponderance of polymorphonuclear cells. There were also, however, many large mononuclear cells, usually with loosely arranged, palely stained nuclei. Red cells were practically absent.

On the *fifth day* the fluid had practically disappeared, and all that remained was a layer of fibrin. This was on the peritoneum, but especially on the diaphragm and on the surface of the liver and spleen. In the meshes of this fibrin were many pigment granules either free or enclosed within phagocytes.

Evidently it is not possible to compare the output from the thoracic duct in these instances with what it was before the injections were made. But from a knowledge of the findings in a great many cases it is possible to assume that certain types of cells were present in certain relative proportions, and to consider wide variations from this standard as unusual and perhaps as a consequence of certain factors and agencies employed in the instance in question.

The most extensive and detailed enumeration of the cell content of the lymph from the thoracic duct has been reported from this laboratory by Rous.[1] His report is based upon a careful consideration of the number and types of cells found in the lymph from twenty-three dogs in which, as far as could be ascertained, no complicating conditions existed.

[1] Rous, Peyton, *Jour. Exper. Med.*, 1908, x, 537.

My counts of the cells of the lymph can well be compared with the standard established by the findings of Rous. The observations of Delamere, and of Biedl and Decastello, as well as my own in a previous set of experiments, are also taken into account in this connection. Rous[2] states: "Mast cells are not a constituent of the lymph of the normal dog. Polymorphonuclear neutrophiles are only present as a result of blood admixture. Lymphocytes, by which are meant non-granular cells with a round or oval nucleus smaller than an entire polymorphonuclear neutrophile from the same animal, form an average of 87.6 per cent. of the cells." The percentage goes as low as 69.8 per cent. and as high as 96.8 per cent. "Typical transitional forms are rare in the lymph. . . . Large mononuclear cells—non-granular mononuclear elements larger than the lymphocyte as above defined—average 5.2 per cent. of the lymph's leucocytes." From the differences in the cell counts in individual dogs it is evident that in normal animals great variations from these average percentages occur. Since the variations are normally so great, the proportions of the cells must be altered extremely before we can be sure that a given agency has produced a noteworthy effect.

I have made a total and differential count of the cells in the lymph of the thoracic duct in each of my cases, the results of which are given in table I. By comparing the data in this table with those already cited, we may draw our conclusions.

If such a table could be made to apply to a single animal over a period of five days, and the test repeated on as many animals as are here represented, it would be very easy to form rather definite conclusions as to the types of the cells and the degree of variation in the cell formula of the lymph under varying conditions and agencies. Such procedures, however, do not seem to be practicable.

We can do little more than compare the relative proportions of the various elements in different animals and the approximate rates of flow.

The *small mononuclear* cells, which primarily constitute the bulk of cells in the lymph, here show a range of from 63.8 per cent. to 92.4 per cent. These extremes cannot be said to be far from the

[2] Rous, Peyton, *loc. cit.*

TABLE I.

Total and Differential Counts of Cells in the Thoracic Duct at Varying Intervals after Injection of Oil of Turpentine into the Peritoneal Cavity of Dogs. Each Set of Counts Represents a Different Animal. Eleven Animals Were Used.

After injection.	Small mononuclears.		Large mononuclears.		Polymorphonuclear neutrophils.		Eosinophils.		Transitionals.		Total counted.	Total per c.mm.	Flow in 10 min. in c.c.
	No. of cells.	Per cent.	No. of cells.	Per cent.	No. of cells.	Per cent.	No. of cells.	Per cent.	No. of cells.	Per cent.			
4 hrs.	476	84.5	59	10.4	7	1.2	9	1.6	12	2.1	563	8,650	7.0
6 hrs.	403	79.6	77	15.2	6	1.1	3	0.56	16	3.1	505	5,500	12.5
36 hrs.	417	73.5	105	18.5	12	2.1	7	1.2	26	4.5	567	9,975	9.5
2d day	348	71.6	91	18.7	27	5.5	2	0.4	18	3.7	486	7,500	14.0
2d day	240	63.8	73	19.4	19	5.0	12	3.2	32	8.5	376	10,400	7.0
3d day	427	73.7	96	16.6	7	1.2	6	1.0	42	7.2	578	9,225	13.0
3d day	318	70.4	85	18.8	21	4.6	4	0.9	23	5.0	451	12,600	13.5
4th day	409	83.4	28	5.7	26	5.3	18	3.7	9	1.8	490	11,900	8.0
4th day	381	89.4	19	4.4	14	3.3	3	0.7	9	2.1	426	19,100	11.0
5th day	412	91.9	15	3.3	5	1.1	11	2.4	5	1.1	448	6,475	7.5
5th day	341	92.4	13	3.5	9	2.4	2	0.54	4	1.09	396	11,500	5.0

percentages taken as a standard, although the lowest count is below the minimum percentage obtained by Rous.

The variations of percentage with reference to the effusion are significant. At first when the amount of effusion was small and the cells contained in it were few, the percentage of mononuclear cells in the lymph was relatively high. Later, as the *effusion* became characterized by mononuclear cells of this type, the percentage in the *lymph* diminished. This is, perhaps, not what we should expect if we were to take the view that the cells of the effusion are supplied from the blood, and that the supply in the blood is maintained by the cells coming from the thoracic duct. But even from this point of view it seems possible to explain the findings.

The first effect of the injection of the turpentine is a demand upon the protective forces of the body. This is evidenced by the prompt pouring out of a fluid that acts as a diluent and perhaps as an antagonist chemically to the irritant agent. Simultaneously with this there is an increased activity on the part of the tissues or organs which furnish the cellular elements. Hence a high percentage of the elements in the lymph at this time consists of cells that are about to be passed into the blood and later into the peritoneal cavity. But

there is no doubt that many of the cells go to the seat of activity without passing through the ordinary channels. This is especially true later in the process, and accounts for the continued relative increase in mononuclear cells in the effusion after the relative decrease in the thoracic duct. Later in the process the percentage of the small mononuclear cells in the lymph rises again to a high mark, due perhaps to the reverting of the supply into the usual channel and to the taking up of many of these cells from the peritoneal cavity. That the mononuclear cells are taken up from the peritoneal cavity is evidenced by the great numbers of poorly stained, loosely formed, and misshaped cells of this type that are seen in the smears from the lymph at this time.

The *large mononuclears* maintain a ratio that is practically the reciprocal of that of the small mononuclears. The percentage of the large mononuclear cells in the lymph is high immediately before and during the time when their number in the peritoneal fluid is greatest. Late in the process, however, the ratio of the large mononuclear cells in the lymph to those in the peritoneal fluid is relatively low.

This may indicate that the supply of the large mononuclears is not immediately restored or that these cells do not return to the thoracic duct once they have been passed into the serous cavity. However, the number of these cells that show the effects of soaking in the fluid, as evidenced by loss of definite outline, loose chromatin, and pale staining, would seem to indicate that many of them are returned to the thoracic duct. Although the percentages of the large mononuclear cells in the lymph in my animals varied greatly, they did not go beyond the limits found in normal conditions.

Polymorphonuclear neutrophils constituted from 1.1 per cent. to 5.5 per cent. in this series of counts. This ratio is higher than the standard adopted, although Rous shows one instance of 11 per cent. with apparently only a slight admixture of blood. However, in my animals the number of polymorphonuclears apparently bears no relation to the other features of the condition. It would seem, then, that whatever may be the number of this type of cells in the lymph of the thoracic duct, it is not determined by the number furnished to the blood, or by the number removed from an effusion

containing a large number of these cells. This may be taken as another indication that the thoracic duct is not a highway for the polymorphonuclear neutrophils.

The relation of the *eosinophils* to the process under study appeared to be no greater than that of the polymorphonuclear cells just considered. My ratios ranged from 0.4 to 3.7 per cent.

The number of *transitional cells* in the lymph of my animals was much greater than was anticipated in view of what has been stated regarding their presence in the lymph of normal dogs. Their numbers range from 1.09 to 8.5 per cent. It may perhaps be significant that in the lymph the increase precedes and accompanies the increase of the polymorphonuclear cells in the effusion. However, the percentage of the transitional cells in the lymph is too low for us to attribute the supply of these cells in the blood to the number received from the thoracic duct. The small number of the cells in the lymph may be merely indicative of the way in which the body draws upon all possible sources of supply in conditions such as have been induced here.

The total cell count per cubic millimeter is variable and in itself does not bear a definite relation to the process. If at times during the course of the process we could compare each case with itself before the process began, perhaps more significant factors would appear. The rate of flow was as variable and as inconsistent, apparently, as the total cell count.

In considering all of these points it must be remembered that many factors, which it is difficult to have exactly alike in each case, have a bearing upon the results. The size and activity of the animal, the nature of the food, and the time it is taken are some of these factors.

In addition to the experiments just reported, others were made. One was suggested by a report of Opie[3] in which in dogs an effusion into one pleural cavity was secured by injecting turpentine into the other. This result was obtained only occasionally. To ascertain if the site giving rise to the effusion is identical with the field of irritation, I opened the peritoneal cavity of a dog, under chloretone anesthesia, and holding the intestines upward toward the diaphragm,

[3] Opie, E. L., *Jour. Exper. Med.*, 1907, ix, 391.

I placed a piece of gauze saturated with turpentine in the right inguinal region. Gauze was used rather than the free turpentine so as to prevent, as much as possible, any spread of the irritant. Holding the irritant in this position a quantity of fluid (fifteen to twenty cubic centimeters) collected within an hour, and it was plainly evident that portions of the serous membrane far distant were pouring out the fluid as well as the field locally irritated. This indicates that some nervous mechanism probably takes part in the process, and this explanation may also hold for the observations of Opie relative to the two pleural cavities.

The disposition of pigment placed within the peritoneal cavity was also considered in this connection. From the extensive investigation by Starling and Tubbey[4] it is concluded that soluble coloring matters are absorbed to the greatest degree from the serous cavity directly into the blood-vessels, the urine showing the color earlier than the lymph in the thoracic duct.

The method by which insoluble pigment is disposed of, and the route the pigment takes have also received much attention. The method established by von Recklinhausen[5] has been modified by more recent observers so that now we may consider that there are at least two general ways by which pigment is removed; namely, (1) through the agency of phagocytes, and (2) by the mechanical aspiration of the substance into the lymph channel by the respiratory movements. This subject, based upon the histology of the tissues concerned, is completely discussed by MacCallum.[6]

In my experiments I desired to see if the phagocytic cells followed the channels of the thoracic duct, but I failed to find a single polymorphonuclear cell containing pigment in the lymph from the thoracic duct. An occasional mononuclear enclosed a few granules of the red pigment previously injected into the cavity. However, pigment in appreciable quantities was found in the retroperitoneal and mediastinal glands. This would indicate that the phagocytes take a shorter and more direct route in depositing the foreign substance in the glands.

[4] Starling, E. H., and Tubbey, A. H., *Jour. Physiol.*, 1894, xvi, 140.
[5] von Recklinhausen, F., *Virchows Arch. f. path. Anat.*, 1863, xxvi, 172.
[6] MacCallum, W. G., *Bull. Johns Hopkins Hosp.*, 1903, xiv, 105.

CONCLUSIONS.

Injection of oil of turpentine into the peritoneal cavity of a dog calls forth immediately an exudate of fluid from the surrounding tissues. The amount of fluid reaches the maximum on the third day and has practically disappeared on the fifth day.

The cell content of this fluid is very small at first, but increases rapidly. The type of the predominating cells also changes. In the early exudate the small mononuclear cells are numerous and the large mononuclears few. Later the number of large mononuclear cells is increased and ultimately the polymorphonuclear cells preponderate. Various forms of atypical cells also occur.

Much of the fluid and many of the cells are removed by way of the thoracic duct. The counts of the cells in the thoracic duct and the estimates based on these indicate that the duct does not remove all of the fluid or cells from the peritoneal cavity. Much fluid is probably taken back directly into the blood, as are many of the cells. Some of the cells make their way to the lymph nodes, while many perhaps undergo complete autolysis in the serous cavity.

The polymorphonuclear cells do not enter the thoracic duct in great numbers. Examination of the lymph from the thoracic duct in the case of my dogs showed the types of cells that are usually found there.

The variation in small mononuclear cells is so related to the cell content of the peritoneal effusion as to indicate that the supply in the blood is maintained from this source. The form and staining qualities of the cells indicate that many of the small mononuclear cells are returned to the thoracic duct.

The ratio of polymorphonuclear cells present in the lymph bears no definite relation to the other features of the process. The transitional cells were increased in number and in their ratio to other cells. The increase in the transitional types accompanies an increase in large mononuclear cells and a decrease in small mononuclears.

None of the atypical forms of cells found in the effusion were seen in the lymph.

The number of eosinophils is without apparent relation to the other features of the process.

No polymorphonuclear cells containing the pigment injected were found in the lymph of the thoracic duct, and the number of mononuclear cells containing pigment was small. Much pigment was deposited in the lymph nodes. Detailed and definite conclusions as to the relation between the cells of the lymph and those of the effusion cannot be arrived at satisfactorily without repeated observations on the same animal.

Dr. Warthin examined many of the smears from these cases and frequently controlled the conclusions regarding the various types of cells. Dr. P. F. Morse assisted me frequently with the operative procedures and with the routine counting of the cells.