LYMPH FORMATION AND EDEMA OF THE LIVER WITH EXPERIMENTAL NEPHRITIS PRODUCED BY CANTHARIDIN.*

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Experimental production of nephritis has offered a means of explaining the pathogenesis of nephritis and its associated phenomena not afforded by methods applicable to patients suffering with the disease. The possibility that changes in the liver have a part in the functional disturbances which accompany nephritis has been suggested by the occurrence of edema of the liver and gall bladder in association with nephritis produced by a substance capable of causing acute nephritis in man, namely, cantharidin. An attempt to analyze the factors upon which this hepatic edema depends has shown that the poison exerts an important influence upon the production and flow of lymph. Experiments with other renal poisons, as yet incomplete, have shown that they exert an important influence both upon the liver and upon the flow of lymph. The present article will be limited to a discussion of the changes caused by cantharidin.

The experimental studies of Heidenhain[1] upon lymph formation define two groups of substances which increase the production of lymph. Heidenhain's lymphagogues of the first order include a variety of protein substances such as peptone, extracts from the tissues of certain invertebrates, namely, extract of muscle of crab, extract of head and body of the leech, egg albumen, and other substances. When injected into the circulation of the dog, they cause an acceleration of the flow of lymph from the thoracic duct. This increase begins immediately after injection and lasts an hour or more; the lymph after injection contains an increased quantity of protein though its content in inorganic salts remains unchanged. Ten c.c. of an aqueous extract of crab meat may increase the flow of lymph sixfold and increase its protein content from 4.06 to 5.57 per cent.; a solution of peptone causes an even greater acceleration of the flow of lymph. Heidenhain's lymphagogues of the second order are crystalloid sub-

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[1] Heidenhain, R., *Arch. f. d. ges. Physiol.*, 1891, xlix, 209.

stances; among those which he tested were grape sugar, sodium chloride, and sodium sulphate. These substances injected into the circulating blood may increase the flow of lymph from the thoracic duct and this lymph contains less protein than the normal lymph from the thoracic duct. Heidenhain cites experiments which indicate that the increased flow caused by lymphagogues of the first order is dependent upon the vital activity of the endothelial lining of the blood-vessels, whereas the action of crystalloids is dependent upon well known physical forces produced by changes in the concentration of the blood and tissue fluids. Lymphagogues of the first order cause no increase of urinary excretion, whereas crystalloids increase the flow of urine.

Starling[2] found that increase of lymph flow from the thoracic duct caused by peptone or by decoction of the muscle of crayfish may be almost completely prevented by ligation of the portal lymphatics. He attributes the entire increase to lymph derived from the liver and suggests that the small increase of flow which occurs after ligation of the portal lymphatics is referable to flow through those lymphatic channels which accompany the hepatic vein. Starling has found an increase of pressure in the portal vein following injection of so called lymphagogues of Heidenhain's first order, but since increased lymph flow continues after portal pressure has fallen, he believes that increased permeability of the capillaries of the liver has an important part in bringing about increased flow of lymph.

The capillaries of the liver, according to Starling, are more permeable than those in other tissues. Lymph obtained from the liver contains approximately from 6 to 8 per cent. of solids, whereas lymph from the intestine contains from 4 to 6 per cent., and lymph from the extremities only from 2 to 4 per cent. The capillaries of the liver are extremely sensitive to pressure, an elevation of pressure in the hepatic vein equal to 200 mm. of water produced by obstruction of the vena cava above the diaphragm causing an enormous flow of lymph derived from the liver.

The permeability of the hepatic capillaries demonstrated by the studies of Heidenhain, Starling, and others, gives significance to the occurrence of edema of the liver produced by cantharidin. This substance, it is well known, produces changes in the kidney accompanied by albuminuria. At the end of one hour after the injection of from 0.005 to 0.02 gm. of cantharidin into rabbits, Cornil and Brault[3] found an accumulation of material containing nucleated cells within the capsular space of the Malpighian bodies of the kidney. Later, according to their observations, cells lining the capsule are swollen and undergo desquamation. The cells of the convoluted tubules become swollen and their protoplasm is granular and vacuolated; within the lumina there is granular debris. With poisoning caused by repeated administration of small doses of cantharidin, round cells accumulate about the arteries. Eliaschoff[4] observed similar changes; in the capsular space were albuminous precipitate and cells which were regarded as swollen white corpuscles; swelling and desquamation of the capsular epithelium were found. The renal tubules exhibited degenerative changes.

[2] Starling, E. H., *Jour. Physiol.*, 1894, xvi, 224; 1894–5, xvii, 30.
[3] Cornil and Brault, Études sur la pathologie du rein, Paris, 1884, 149.
[4] Eliaschoff, I., *Virchows Arch. f. path. Anat.*, 1883, xciv, 323.

In white rats poisoned with cantharidin, Welch[5] found that cells accumulate in the capsular space. He does not regard these cells as swollen leucocytes which have emigrated from the blood-vessels nor as desquamated epithelial cells, but thinks that they are cells of the convoluted tubules which have been pushed back into the glomerular space. Lyon[6] has observed the same change; he has noted that the capsular space is devoid of contents in animals which survived longer than ten hours, the tuft filling it almost completely. Lyon found advanced degeneration in the tubules; the ascending limb of the loop of Henle may exhibit coagulative necrosis. The convoluted tubules are dilated and the inner parts of the cells undergo disintegration, leaving a basal part containing a nucleus which may stain deeply. The cells of the collecting tubules are swollen and granular, particularly in the larger tubules, and have frequently undergone desquamation into the lumen. Polynuclear leucocytes are often found between these cells. Casts are formed within the collecting tubules, particularly in the medullary rays. With less acute poisoning, lymphocytes may collect about blood-vessels.

Schlayer and Hedinger,[7] and more recently Pearce, Hill, and Eisenbrey,[8] using physiological methods have described experiments which they believe indicate the occurrence of two types of experimental nephritis, one caused by potassium chromate or uranium nitrate and referable to changes in the tubules of the kidney, and the other caused notably by cantharidin or arsenic and referable to changes in the glomeruli. Alteration of glomeruli is demonstrable by vascular reactions rather than by anatomical changes in the capillaries or epithelium of these structures. Animals with nephritis following the administration of potassium chromate or uranium nitrate eliminate an increased quantity of urine and exhibit a variety of reactions which demonstrate that the blood-vessels of the kidney retain their normal excitability to certain stimuli; the oncometer shows that transient sensory stimulation or administration of adrenalin causes the blood-vessels to contract with somewhat abnormal activity, whereas caffein or strong salt solution causes dilatation somewhat greater than that which occurs with administration of the drug in normal animals; phloridzin causes the polyuria and glycosuria observed after its administration to normal animals. In animals which have received cantharidin or arsenic, similar tests applied within from four to eight hours show that the vascular apparatus of the kidney has undergone profound alteration. A diminished quantity of urine is excreted; contraction and dilatation of the vessels under the conditions which have been named are diminished or absent; phloridzin causes no polyuria and no glycosuria.

After the injection of from 0.013 to 0.026 of a gram of cantharidin dissolved in ethyl acetate (one to two cubic centimeters of a solution containing 0.2 of a gram of cantharidin in fifteen cubic

[5] Welch, W. H., *Tr. Assn. Am. Phys.*, 1886, i, 171.

[6] Lyon, G., *Jour. Path. and Bacteriol.*, 1904, ix, 400.

[7] Schlayer and Hedinger, *Deutsch. Arch. f. klin. Med.*, 1907, xc, 1.

[8] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., *Jour. Exper. Med.*, 1910, xii, 196.

centimeters of ethyl acetate) into dogs ranging in weight from 2.5 to 10 kilos, I have found that the changes observed after death are fairly constant. Small animals are killed by 0.013 of a gram of cantharidin but larger animals survive this dose. Death with the hepatic changes which will be described may occur within five hours. The kidneys are slightly enlarged, succulent, and deep red throughout; the medullary rays are conspicuous, at times, as gray markings. Microscopical examination shows the profound alterations in the convoluted tubules described by Welch, Lyon, and others. Particularly noteworthy are the consequent changes within the glomeruli. The capsular space is dilated and contains both granular material, consisting of albuminous precipitate and hyaline droplets, and large nucleated cells, often in excellent preservation. The protoplasm of such cells is identical in appearance with that of the cells which compose the adjacent convoluted tubules. Their nuclei are often irregular in shape; at times the nuclei stain intensely, at times they remain almost unstained. Favorable sections passing through the junction of a glomerular capsule and its convoluted tubule show that the cells within the capsule are continuous with those of the tubule, which occasionally project as a rounded mass without obvious lumen through the neck of the capsular space. The epithelium of the capsule and of the glomerulus exhibits no alteration.

Coincident with the changes which occur in the kidneys, there are noteworthy changes in the liver and gall bladder. The liver is turgid and homogeneously deep red without lobular markings. Blood in great abundance escapes from its cut surface. The gall bladder is swollen and bluish gray in consequence of edema of its wall which may be two or three millimeters in thickness. The cellular tissue between the gall bladder and the liver is conspicuously edematous. Widely dilated lymphatics, often 1.5 millimeters in diameter, accompany the structures contained in the portal spaces and pass downward from the root of the liver through the gastrohepatic omentum to the regional lymphatic nodes which are situated at the base of the duodenal mesentery and in the gastrohepatic omentum along the upper margin of the splenic arm of the pancreas. Similarly dilated lymphatics connect adjacent nodes. The afferent lymphatics of the nodes usually contain blood tinged lymph and

end upon the surface of the node of which the peripheral sinus and medulla are stained red.

The lymphatic nodes which become swollen and edematous are as follows: (1) A large node situated at the base of the duodenal mesentery in contact with the superior mesenteric vein immediately below its junction with the splenic. (2) One, two, or occasionally more smaller nodes situated in the duodenal mesentery between its base and the duodenal arm of the pancreas; these nodes are occasionally in almost immediate contact with a group of large nodes at the base of the cecum. (3) From two to six lymphatic nodes situated in contact with the splenic vein in the mesenteric fold which is attached to the superior margin of the splenic arm of the pancreas. The largest of these nodes is that which is nearest the liver; it is in almost immediate contact with another large node, i. e., (1), which is adjacent to the superior mesenteric vein.

Microscopic examination of the liver demonstrates that edema of the gall bladder is part of a general edema of the liver. The tissue of the portal spaces is distended and its interstices contain albuminous coagulum; lymphatic vessels are dilated. There is similar edematous distension of the tissues above the central and sublobular veins. Dilated lymphatics contain coagulated lymph in which red blood corpuscles are numerous. Within the parenchyma of the lobules the endothelium of the capillaries is separated by wide spaces from the adjacent columns of liver cells, and in these spaces red blood corpuscles are numerous.

Edema of the gall bladder affects the serous more than other coats of the wall. In the serous coat and in the loose tissue in contact with the under surface of the liver are immensely dilated lymphatics which are constricted at intervals by circular valve-like folds.

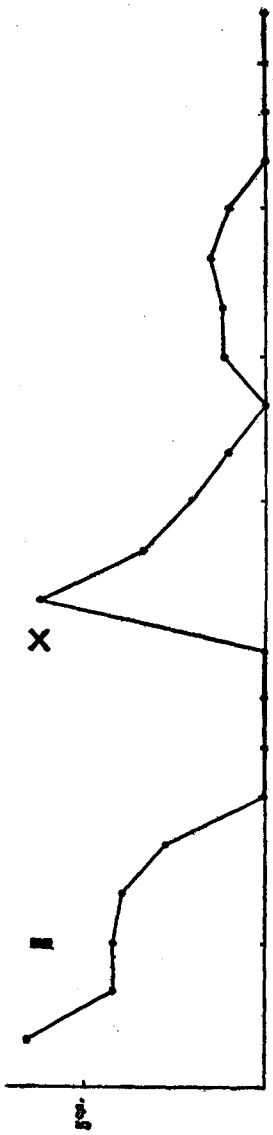
Enlargement of the regional lymphatic nodes of the liver is, in considerable part, the result of immense dilatation of the sinuses and especially of those within the medulla. Here red blood corpuscles, lymphocytes and large mononuclear cells are abundant. Cells showing various stages of disintegration are numerous; some have lost their nuclei whereas others contain deeply stained nuclear fragments. Within such sinuses, fibrinous coagulum forms a network

with fine meshes. It is obvious that fibrin formation has begun about injured cells, many of which still retain the characters of large mononuclear cells, for from these cells radiate in every direction fibrils of fibrin. In some instances, a pale, ill defined nucleus is surrounded by a radiating mass of fibrils which appear to have replaced the protoplasm of the cell. Within the medullary cords and in the cortex of the nodes, destruction of cells is further shown by abundant nuclear fragmentation occurring with greatest frequency in large cells with abundant protoplasm. In the germinating centers of the cortex, necrosis of cells with fragmentation of nuclei is particularly advanced.

Observations of Starling, which have shown that the increased flow of thoracic lymph caused by the action of Heidenhain's lymphagogues of the first order, namely, peptone, decoction of muscle from certain invertebrates such as crab, crayfish, etc., is derived from the liver, have suggested that edema of the liver caused by cantharidin is referable to increased flow of lymph from the liver. It is noteworthy, on the contrary, that administration of the substances employed by Heidenhain is not followed by edema. To determine the effect of cantharidin upon the flow of lymph from the thoracic duct, a cannula has been inserted into the duct exposed near its termination at the junction of the subclavian and jugular veins, and the flow of lymph has been measured during periods of ten minutes. Animals have been etherized during the entire course of the experiment which has been limited by the death of the animal to a period not exceeding three hours. At the end of this time, edema of the gall bladder has been repeatedly well advanced.

Diminution of the flow of thoracic lymph usually occurs within a period of from twenty to thirty minutes after intramuscular injection of cantharidin accompanied by gentle kneading of the injected muscle. In the two following experiments, the flow of lymph from the thoracic duct has been retarded and edema of the gall bladder has been noted at the end of two and of three hours respectively.

Experiment 1.—Dog, weighing 10,800 gm. A cannula was inserted into the thoracic duct; after observation of the lymph flow during 30 minutes cantharidin, 0.02 gm., was injected into the muscles of the thigh (text-figure 1).



TEXT-FIG. 1. Experiment 1, showing the flow of lymph from the thoracic duct after injection of cantharidin. Injection of cantharidin in this and in subsequent figures is indicated by a short heavy black line. At X the abdomen was opened and the intestines were disturbed.

Time.	Lymph in c.c. per 10 min.	Remarks.
12:40–12:50 P. M.	6.65	
12:50– 1:00 P. M.	4.30	
1:00– 1:10 P. M.	4.35	At 1:10 cantharidin, 0.02 gm., intramuscularly.
1:10– 1:20 P. M.	4.00	
1:20– 1:30 P. M.	2.70	
1:30– 1:40 P. M.	0.05	
1:40– 1:50 P. M.	0.02	
1:50– 2:00 P. M.	0.07	
2:00– 2:10 P. M.	0.01	
2:10– 2:20 P. M.	6.34	Abdomen opened and intestines disturbed.
2:20– 2:30 P. M.	3.40	Lymph is tinged with blood.
2:30– 2:40 P. M.	2.05	
2:40– 2:50 P. M.	1.10	
2:50– 3:00 P. M.	0.20	
3:00– 3:10 P. M.	1.20	
3:10– 3:20 P. M.	1.25	
3:20– 3:30 P. M.	1.50	
3:30– 3:40 P. M.	1.00	
3:40– 3:50 P. M.	0.30	
3:50– 4:00 P. M.	0.10	
4:00– 4:10 P. M.	0.10	
4:10– 4:20 P. M.	0.05	

The animal was killed at 4:27 P. M., 3 hours and 17 minutes after injection of cantharidin.

Autopsy.—The liver is red and the gall bladder edematous; conspicuous dilated lymphatics extend from the liver to the lymphatic nodes situated in the duodenal mesentery near its base and along the upper margin of the splenic arm of the pancreas. Microscopical examination of the liver shows edema, escape of red blood corpuscles, and dilatation of lymphatics. These nodes are enlarged and, with the surrounding tissue, edematous. The kidney is cloudy in appearance; the medullary rays are conspicuous.

Experiment 2.—A cannula was placed in the thoracic duct of a dog weighing 7,600 gm. A record of the quantity of lymph obtained during periods of 10 minutes is given below. After noting the flow during 30 minutes, 0.02 gm. of cantharidin was injected into the muscles of the thigh.

Time.	Lymph in c.c. per 10 min.	Remarks
11:05–11:15 A. M.	0.8	Lymph opaque and white.
11:15–11:25 A. M.	1.3	
11:25–11:35 A. M.	2.5	At 11:25 cantharidin, 0.02 gm., was injected.
11:35–11:45 A. M.	1.3	
11:45–11:55 A. M.	2.1	Lymph tinged with blood.
11:55–12:05 P. M.	1.3	

12:05–12:15 P. M.	1.0	
12:25–12:35 P. M.	0.6	
12:25–12:35 P. M.	0.6	
12:35–12:45 P. M.	0.35	
12:45–12:55 P. M.	0.9	
12:55– 1:05 P. M.	1.5	Lymph yellow and almost clear.
1:05– 1:15 P. M.	1.75	
1:15– 1:25 P. M.	2.1	
1:25– 1:35 P. M.	1.45	Death occcurred at 1:35 P. M.

Autopsy.—The liver is homogeneously deep red. The wall of the gall bladder is thickened by edema and there is edema of the gastrohepatic omentum, of the duodenal mesentery, and of the tissues about the ileocecal and mesenteric lymphatic nodes. Microscopical examination of the liver shows edema with escape of red blood corpuscles and dilatation of lymphatics. The hepatic regional lymphatic nodes in the duodenal mesentery near its base and along the upper margin of the splenic arm of the pancreas are enlarged and edematous. The kidney is deep red; the medullary striæ are conspicuous. Urine in the bladder contains no albumin and remains clear when boiled and acidified; it causes an atypical reduction of Fehling's solution but does not ferment.

In these experiments, administration of cantharidin has been followed by almost complete cessation of the flow of lymph. Although it has been possible to follow the changes which are caused by the poison during only three hours, advanced edema of the liver and gall bladder has been present. Such edema has been almost constantly observed when more prolonged action of the drug has caused death within from five to fifteen hours. The histological character of the edematous lesions has been described.

In experiment 1 at 2:10 P. M. the abdomen was opened and the intestines were manipulated in order to examine the bile ducts and the duodenal mesentery. This disturbance of the intestines was immediately followed by temporary discharge of a considerable quantity of thoracic lymph. Starling[9] has observed that pressure upon the intestines liberates abundant lymph. This temporary flow in experiment 1 serves to demonstrate that the otherwise almost total cessation of lymphatic flow is not the result of coagulation or other obstruction within the cannula or adjacent injured thoracic duct.

In the following experiment diminution of flow from the thoracic duct has followed almost immediately the administration of cantharidin but diminution of flow has been irregular and temporary; some edema of the gall bladder has been produced.

[9] Starling, E. H., *loc. cit.*, p. 224.

Experiment 3.—Dog, weighing 7,800 gm. A cannula was inserted into the thoracic duct. The foregoing experiment was repeated.

Time.	Lymph in c.c. per 10 min.	Remarks.
11:00–11:10 A. M.	4.9	Lymph grayish white.
11:10–11:20 A. M.	6.5	
11:20–11:30 A. M.	7.0	At 11:30 cantharidin, 0.02 gm., intramuscularly.
11:30–11:40 A. M.	3.4	
11:40–11:50 A. M.	1.7	Lymph faintly red.
11:50–12:00 A. M.	5.9	Lymph reddish in color.
12:00–12:10 P. M.	2.8	
12:10–12:20 P. M.	3.8	Lymph well marked yellow.
12:20–12:30 P. M.	4.5	
12:30–12:40 P. M.	2.1	
12:40–12:50 P. M.	4.4	Lymph deep yellow.
12:50– 1:00 P. M.	4.3	Lymph deep brownish yellow.
1:00– 1:10 P. M.	4.9	
1:10– 1:20 P. M.	4.3	
1:20– 1:30 P. M.	5.4	
1:30– 1:40 P. M.	6.3	
1:40– 1:49 P. M.	4.9	Died.

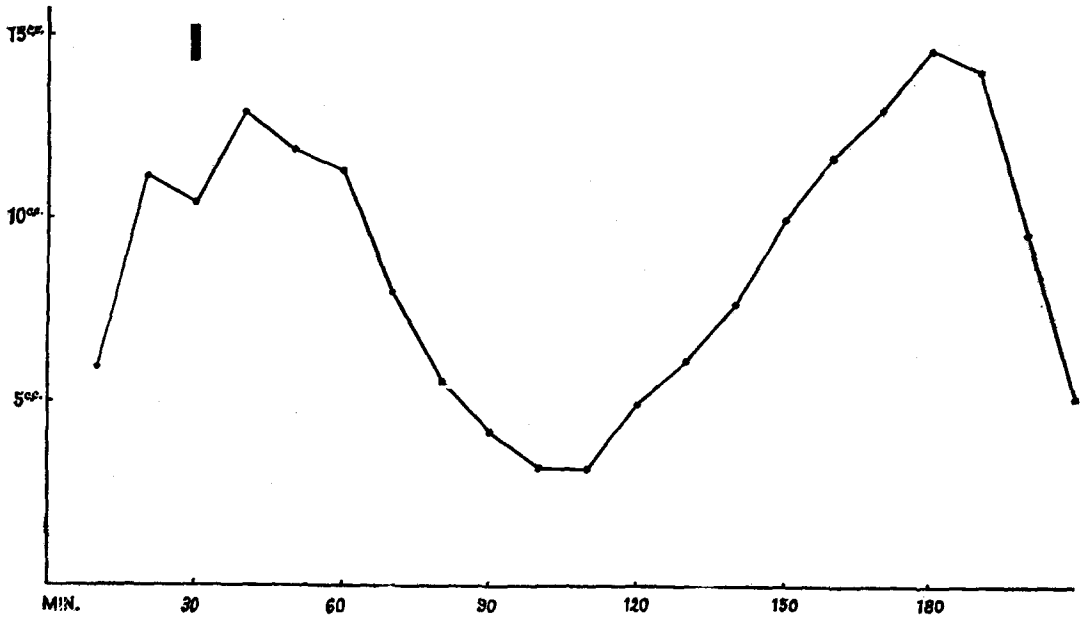
Autopsy.—The liver shows no noteworthy abnormality; the gall bladder is edematous in patches but is not uniformly swollen. The regional lymphatic nodes are swollen, red, and in part edematous. Dilated lymphatics pass from the liver to these nodes.

The urine from the urinary bladder contains a moderate amount of albumin. Reduction indicates the presence of 1.17 per cent. of sugar; the urine ferments.

In two experiments which will be described, there has been temporary diminution of lymph flow following injection of the drug, whereas in a third experiment (experiment 6) no diminution has occurred; yet in all of these experiments after about one and a half hours, there has been a greatly increased flow of thoracic lymph. With this active flow of lymph, no edema of the gall bladder or liver has occurred.

Experiment 4.—Dog, weighing 13,400 gm. A cannula was inserted into the thoracic duct with considerable difficulty (text-figure 2).

Time.	Lymph in c.c. per 10 min.	Remarks.
1:50–2:00 P. M.	5.8	Lymph grayish white.
2:00–2:10 P. M.	11.2	
2:10–2:20 P. M.	10.4	At 2:20 to 2:21 cantharidin, 0.052 gm., intramuscularly.
2:20–2:30 P. M.	12.85	



TEXT-FIG. 2. Experiment 4, showing the flow of lymph from the thoracic duct after injection of cantharidin.

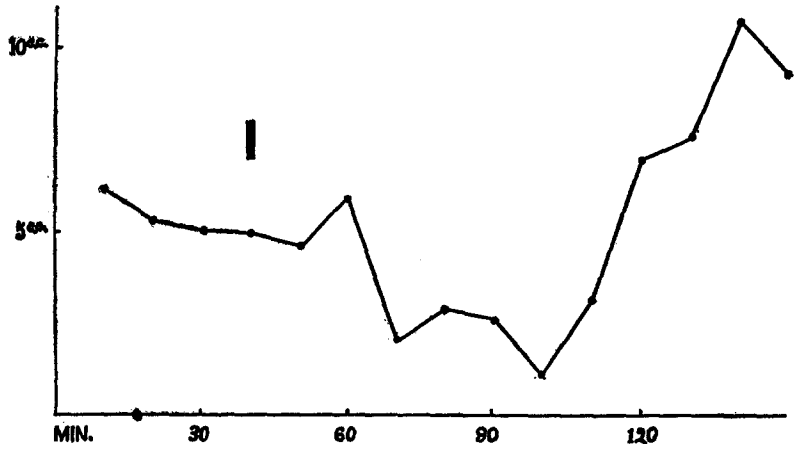
2:30–2:40 P. M.	11.7	
2:40–2:50 P. M.	11.3	Lymph blood tinged.
2:50–3:00 P. M.	7.9	
3:00–3:10 P. M.	5.5	
3:10–3:20 P. M.	4.2	
3:20–3:30 P. M.	3.2	
3:30–3:40 P. M.	3.2	
3:40–3:50 P. M.	4.8	
3:50–4:00 P. M.	6.1	
4:00–4:10 P. M.	7.6	Lymph deep red.
4:10–4:20 P. M.	9.9	
4:20–4:30 P. M.	11.65	
4:30–4:40 P. M.	12.9	
4:40–4:50 P. M.	14.6	
4:50–5:00 P. M.	14.0	
5:00–5:10 P. M.	9.6	
5:10–5:20 P. M.	5.2	Died.

Autopsy.—Liver is deep red. There is no edema of the gall bladder.

Experiment 5.—Dog, weighing 12,600 gm. A cannula was inserted into the thoracic duct (text-figure 3).

Time.	Lymph in c.c. per 10 min.	Remarks.
12:50–1:00 P. M.	6.2	Lymph opaque, white.
1:00–1:10 P. M.	5.3	
1:10–1:20 P. M.	5.0	
1:20–1:30 P. M.	4.9	At 1:30 cantharidin, 0.052 gm., intramuscularly.
1:30–1:40 P. M.	4.6	
1:40–1:50 P. M.	5.8	
1:50–2:00 P. M.	2.0	
2:00–2:10 P. M.	2.8	
2:10–2:20 P. M.	2.6	
2:20–2:30 P. M.	1.05	
2:30–2:40 P. M.	3.1	Lymph faintly blood tinged.
2:40–2:50 P. M.	7.1	
2:50–3:00 P. M.	7.6	Lymph blood tinged.
3:00–3:10 P. M.	10.7	Lymph yellowish.
3:10–3:20 P. M.	9.3	Lymph deeper yellow.
3:20–3:30 P. M.	4.2	Lymph bright yellow. Death.

Autopsy.—There is no edema of the gall bladder.



TEXT-FIG. 3. Experiment 5, showing the flow of lymph from the thoracic duct after injection of cantharidin.

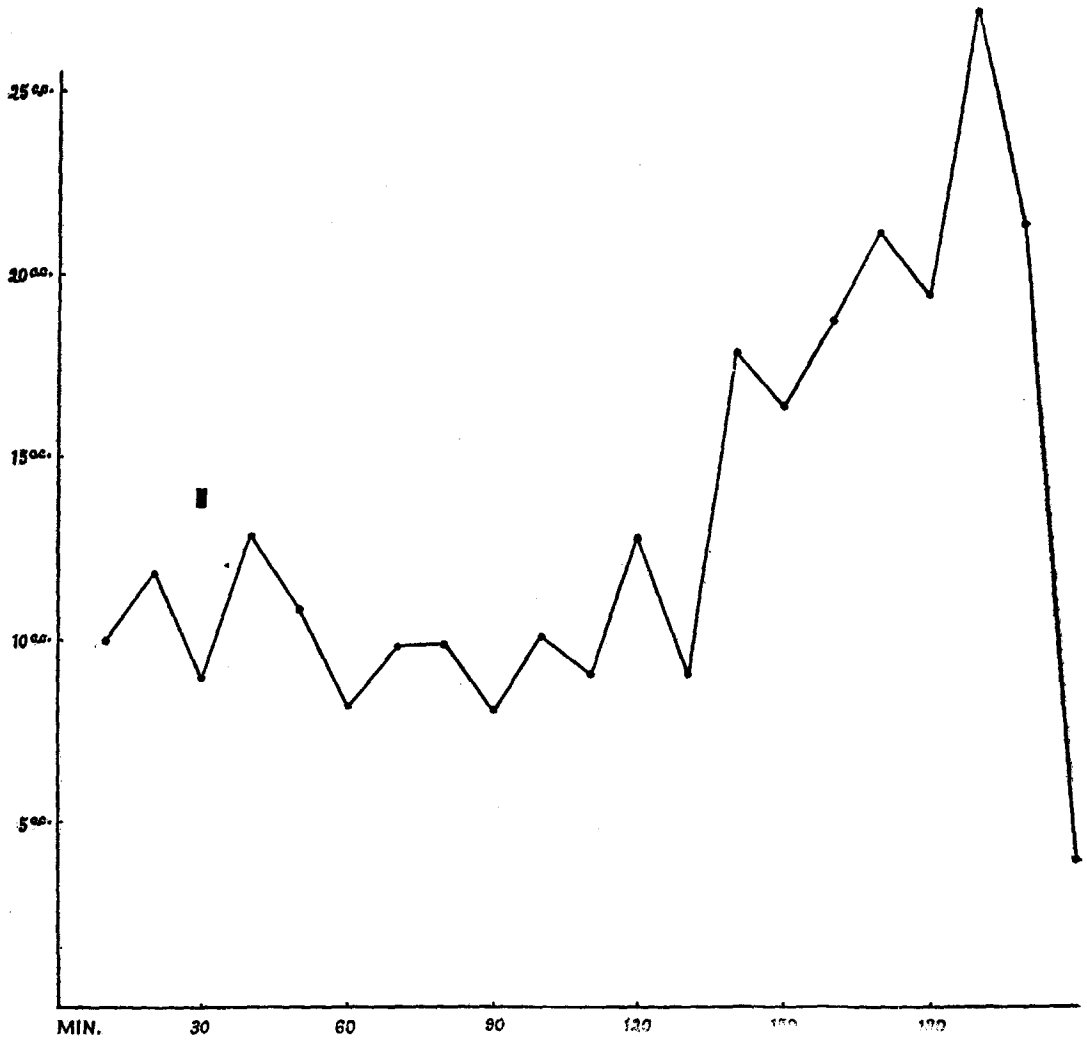
In the following experiment, injection of cantharidin, as in experiments 4 and 5, caused a well marked increase in the flow of lymph, but this increased flow was not preceded by a period during which the flow was diminished.

Experiment 6.—Dog, weighing 18,600 gm. A cannula was placed in the thoracic duct (text-figure 4).

Time.	Lymph in c.c. per 10 min.	Remarks.
12:10–12:20 P. M.	10.0	Lymph opaque, white, slightly blood tinged.
12:20–12:30 P. M.	11.8	
12:30–12:40 P. M.	8.9	At 12:40 cantharidin, 0.02 gm., intramuscularly.
12:40–12:50 P. M.	12.8	
12:50– 1:00 P. M.	10.7	
1:00– 1:10 P. M.	8.3	
1:10– 1:20 P. M.	9.8	
1:20– 1:30 P. M.	9.8	
1:30– 1:40 P. M.	8.2	
1:40– 1:50 P. M.	10.3	Lymph blood stained.
1:50– 2:00 P. M.	9.2	
2:00– 2:10 P. M.	12.9	
2:10– 2:20 P. M.	9.2	
2:20– 2:30 P. M.	18.0	
2:30– 2:40 P. M.	16.5	
2:40– 2:50 P. M.	18.8	
2:50– 3:00 P. M.	21.3	
3:00– 3:10 P. M.	19.5	
3:10– 3:20 P. M.	27.3	
3:20– 3:30 P. M.	21.4	
3:30– 3:35 P. M.	4.0	Died at 3:35.

Autopsy.—The liver is moderately deep red. There is no edema of the gall bladder. The regional lymphatic nodes are enlarged and have red centers. Dilated lymphatics pass from the liver to these nodes.

The experiments which have been described are confirmed by others in which similar results have been obtained. The effect of cantharidin upon the flow of lymph from the thoracic duct is as follows: (a) Intramuscular injection of a fatal dose (0.02 of a gram) is usually followed by well marked diminution of the flow, which is evident after from twenty to thirty minutes and reaches a minimum at the end of about one hour; the lymph is tinged with blood. No subsequent rise occurs and there is edema of the liver recognizable within from two to three hours after injection. (b) In a second group of experiments occurring more frequently with larger doses of cantharidin (0.05 of a gram), an increased flow of lymph follows the retardation which has been noted or occasionally occurs without a preceding diminution of output. This increased



TEXT-FIG. 4. Experiment 6, showing the flow of lymph from the thoracic duct after injection of cantharidin.

flow, occurring after an interval approximately from one and a half to two hours after injection of the drug, may greatly exceed the normal. The lymph is no longer tinged with blood but assumes a yellow color and coagulates slowly.

Within the period of observation permitted by the experiment larger doses more frequently demonstrate the lymphagogue action

of the poison. With smaller doses the flow of lymph does not recover from its primary depression. Dilatation of lymphatics and edema of the liver and gall bladder occur during the time when flow of lymph from the thoracic duct is diminished and is almost constantly observed when death is caused by acute poisoning with cantharidin. Edema of the liver furnishes evidence that there is increased lymph formation in the liver before this increased production causes an increased flow from the thoracic duct. Histological examination shows that obstruction to the lymph stream capable of producing simultaneously edema and diminished flow of thoracic lymph occurs within lymphatic channels and notably within the sinuses of the regional nodes of the liver.

Those nodes which receive lymph from the liver are accurately defined by the poison. Their situation has already been described; they become enlarged and their medulla assumes a deep red color. The nodes which are affected are connected with the liver by lines of dilated lymphatics which pass downward from liver and gall bladder to the proximal nodes and hence from one node to the next. These lymphatics contain lymph deeply tinged with blood and their termination in the peripheral sinus of the node is well defined by similar localized discoloration.

Microscopic examination of the regional nodes removed from animals which have died with edema of the liver and gall bladder during the period of diminished flow from the thoracic duct shows that the afferent lymphatics and the sinuses of the node, notably the peripheral sinus, contain fibrin which usually forms a fine network but occasionally occurs in more compact particles. Particularly noteworthy is the relationship of this fibrinous network to cells which have undergone degenerative changes. Cells of which the nuclei are pale and ill defined or wholly lost form centers from which radiate fibrils of fibrin. A similar radiate arrangement of fibrin occurs upon the free surface of endothelial cells still attached to the walls of the sinus.

Within the widely dilated sinuses of the node, cells are very numerous, often closely packed together and connected by the fibrinous network which has been described. Large mononuclear cells similar to those which line the sinuses are particularly numer-

ous and are mingled with lymphoid cells and polynuclear leucocytes, the latter being present in moderate number. Cells exhibiting evidence of degeneration and necrosis, doubtless caused by the poison, are numerous; many exhibit nuclear fragmentation; others have assumed a hyaline appearance and have lost their nuclei. The destructive action of cantharidin is best seen in the germinating centers of the cortex where nuclear fragmentation is usually widespread. The presence of red blood corpuscles outside the capillaries of the liver, within the hepatic lymphatics and in the sinuses of the lymphatic nodes, is further evidence of the injurious action of the poison which appears to affect notably endothelium of the blood-vessels and lymphatics. Phagocytosis of red blood corpuscles by the large mononuclear cells which have been mentioned is actively in progress two to three hours after administration of cantharidin. Clumping of red blood corpuscles upon the surface of these cells may have a part in plugging the sinuses of the regional hepatic nodes. In one specimen masses of red corpuscles were adherent to the surface of mononuclear phagocytes which had taken many red corpuscles into their substance. Such observations show that the cell does not move toward the red corpuscles and ingest it, but attracts the corpuscle and holds it. I have not determined whether the conspicuous yellow color assumed by the lymph from the thoracic duct is referable to destruction of red corpuscles with setting free of hemoglobin or to the presence of bile liberated by the injured liver.

Progressive dilatation of lymphatics and of the sinuses of the lymphatic nodes finally overcomes the obstruction opposed by the fibrinous thrombi which are formed in these channels. Diminished coagulability of the lymph probably explains the cessation of progressive formation of fibrinous plugs. Increased flow of lymph often far exceeding the normal thus follows the temporarily diminished output.

In order to determine the relationship of lymphatic nodes to the changes which occur in the liver, the effect of cantharidin has been tested upon animals from which the regional hepatic nodes have been extirpated. Removal of these nodes may be followed by establishment of collateral lymphatic circulation through channels which

pass directly to the thoracic duct without intervention of lymphatic nodes. Nodes which have been overlooked at the time of operation undergo hypertrophy. Whereas in normal animals fatal cantharidin poisoning has been almost invariably followed by edema of the liver and gall bladder, edema has occurred in only one of three animals in which, after extirpation of the regional nodes, death has followed administration of the drug. In this instance there was edema of the liver, gall bladder, and pancreas, and accumulation of fluid in the peritoneal cavity; a large lymphatic node was found in the gastrohepatic omentum and numerous dilated lymphatics passed to it from the liver. This enlarged node had undergone the changes which have been described, the sinuses being plugged with fibrin.

Experiment 7.—Dog, weighing 9,800 gm. The abdomen was opened and all the lymphatic nodes which could be found in the gastrohepatic omentum, in the duodenal mesentery and near the splenic vein along the upper margin of the splenic arm of the pancreas were removed. On the 22d day after operation, cantharidin, 0.013 gm., was administered intramuscularly; on the following day, 724 c.c. of urine contained 4.16 gm. of fermentable sugar and albumin in abundance; on the next day sugar was still present. On the 26th day cantharidin, 0.006 gm., was administered; 550 c.c. of urine collected during the following day contained 2.43 gm. of sugar and abundant albumin. On the 30th day cantharidin, 0.013 gm., was administered and albuminuria but no glycosuria followed. On the 40th day cantharidin, 0.02 gm., was given; death occurred during the following night. The urine, 330 c.c., contained 3.52 gm. of sugar (Benedict's method), and albumin, determined by Esbach's method, equal to 2.667 gm. per liter.

Autopsy.—The peritoneal cavity contains 23 c.c. of red serous fluid. The liver is deep red; the gall bladder is edematous throughout, the wall measuring 2 to 3 mm. in thickness. Many dilated lymphatics filled with blood stained lymph pass in parallel lines to a firm, deep red lymphatic node 2 cm. in length, situated in the gastrohepatic omentum at the margin of the foramen of Winslow. Two smaller blood tinged nodes are found at the base of the duodenal mesentery. The tissues of the gastrohepatic omentum are edematous. The heart is normal save for edema of the fibrous tissue at the base of two aortic segments accompanied by edema of the segment of the tricuspid valve on the opposite side of the interventricular septum. The thymus is dull red throughout; the adjacent substernal lymphatic nodes are enlarged and red and the tissue about them is edematous.

Microscopical examination shows well marked edema of the liver and pancreas. In many places hepatic cells have separated from one another so that they are no longer arranged in columns. The cells composing acini and islands of Langerhans of the pancreas are similarly separated. The sinuses of lymphatic nodes in the positions which have been mentioned contain fibrin in abundance. Here fibrin often radiates from the surface of necrotic cells.

In the following experiments no large lymphatic nodes escaped the operation planned for their removal, and edema of the liver and gall bladder failed to occur.

Experiment 8.—Dog, weighing 8,000 gm. The operation previously described was repeated. On the 22d day after operation, the animal had gained 1,200 gm. in weight and received cantharidin, 0.013 gm.; 870 c.c. of urine passed during the following day contained 4.61 gm. of sugar. Cantharidin, 0.006 gm., on the 25th day caused no glycosuria. Death followed within 15 hours after the administration of cantharidin, 0.02 gm.; 925 c.c. of urine contained fairly abundant albumin but no sugar.

Autopsy.—The liver is deep red; there is no edema of the gall bladder. Above the splenic arm of the pancreas is a small lymphatic node 0.3 by 0.5 cm. The pancreas is slightly edematous and there is well marked edema of the gastrohepatic omentum in the neighborhood of the lesser curvature of the stomach. Microscopical examination of the liver shows congestion but no edema.

Experiment 9.—Dog, weighing 6,000 gm. The regional hepatic nodes together with other abdominal lymphatic nodes were removed. Cantharidin, 0.013 gm., administered on the 18th day caused death after 40 hours; urine, 820 c.c., collected during the following day contained abundant albumin and 8.04 gm. of sugar (Benedict's method; fermentation not tested), and on the next day the urine (80 c.c.) contained a moderate amount of albumin and 0.18 gm. of sugar (fermentation not tested).

Autopsy.—The liver is pale brownish red; there is no edema of the gall bladder. Dilated lymphatics pass from the liver and uniting form a large trunk which passes into the retroperitoneal tissue and joins the receptaculum chyli. No lymphatic nodes are found at the base of the duodenal mesentery or along the superior margin of the splenic arm of the pancreas. Microscopical examination shows no edema of the liver or pancreas.

The experiments are inconclusive with regard to the effect of the removal of lymphatic nodes upon the action of cantharidin. When, on the one hand, some of the regional nodes escape operative removal (experiment 7), these nodes under the influence of cantharidin may oppose obstruction to the flow of lymph and in consequence edema occurs. When, on the other hand, all nodes are removed, collateral circulation (experiment 9) may permit the lymph to flow into the thoracic duct and no edema follows the administration of cantharidin. Although the number of experiments is too small to define the action of cantharidin in animals deprived of regional hepatic lymphatic nodes, they support the view that occlusion of lymphatic channels, in association with increased lymph formation, is the cause of the edema which follows administration of cantharidin.

The foregoing experiments define the changes which accompany

edema of the liver and gall bladder. Separation of columns of liver cells from adjacent capillaries, the intervening space containing serum and often red blood corpuscles, and separation of liver cells from one another, are manifestations of edema of the liver.

The flow of lymph from the thoracic duct is profoundly modified by cantharidin; after administration of the drug, the flow diminishes or ceases completely. Coincident with cessation and within three hours after administration of the poison, edema of the liver and gall bladder may be well advanced. Edema caused by cantharidin is almost wholly limited to these organs and to the adjacent lymphatic nodes.

Edema of the liver is dependent upon changes in the lymphatics and in the lymph sinuses of the regional lymphatic nodes, the effective change being the formation of fibrinous coagulum within the sinuses. This coagulum often has its origin about cells injured by the poison, shreds of fibrin radiating from partially or completely necrotic cells.

Obstruction interposed by fibrin formations within lymphatic nodes is overcome in a certain number of experiments, and diminished flow from the thoracic duct is followed by an output which may considerably exceed that observed before administration of the poison. Distension of lymphatics in the liver and occurrence of edema during the period of diminished flow indicates that increased formation of lymph occurs in the liver before increased flow of thoracic lymph demonstrates its presence. In one experiment increased flow was not preceded by a period of diminished output; obstruction to outflow from lymphatics failed to occur or was insufficient to diminish the flow of lymph below normal. In this experiment edema of the gall bladder and liver was absent.

A poison which is capable of causing nephritis increases the flow of lymph from the thoracic duct; it acts as a lymphagogue. In the presence of some additional factor, such as obstruction within the regional lymphatic nodes, edema results. These changes are coincident with the production of the renal lesion and are not the result of disturbance of renal function. They furnish, by analogy, support to the view that dropsy in association with nephritis is caused by the action of an irritant which simultaneously injures the kidney and increases the permeability of vessel walls elsewhere in the body.