

Morphological Studies on the Rat Liver and a Biochemical Analysis of the Serum Following Experimental Ligature of the Thoracic Duct*

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Summary

After ligature of the thoracic duct of rats the spaces of *Disse* and *Mall* as well as the lymphatic vessels in the portal tracts and the lymphatic vessels along the Vv. hepaticae dilate. There are necroses of hepatocytes at the periphery of the liver lobules and microscopical findings which are indicative of lymphobiliary fistulae. After 12-15 days the morphological changes regress more or less.

During the 1st day the serum enzyme activities show an obvious increase in LDH, a fairly small rise in GOT and GPT and a decrease in alkaline phosphatase. Thereafter, only insignificant fluctuations, deviating very little from normal GOT, GPT and LDH values, can be observed. The alkaline phosphatase remains decreased during the whole duration of the experiments. The triglycerides, too, show a decrease in comparison with the normal values, whereas the total lipids and the total cholesterol exhibit only small fluctuations, not deviating very much from normal values.

In the case of animals which were observed during a longer period, the wet weight of the liver was lower than that of healthy control animals.

With respect to our findings of a lymphoedema in transplanted livers (1) the aim of the experiments preceding this paper was to find out which role a lymphoedema within the liver plays in the promotion or aggravation of liver diseases. For this reason, we looked for a method which might produce a marked lymphoedema but did not interfere with rejection, cause ischemic lesions, etc.. *Huth et al.* (2, 3) ligated the lymphatic vessels within the liver hilus, but since this procedure does not include the lymphatic vessels accompanying the Vv. hepaticae it is not satisfying.

In order to achieve a more complete lymphoedema within the liver, we ligated the thoracic duct just before its entry into the left angulus venosus at the base of the neck.

Material

308 white male and female Sprague-Dawley rats weighing between 140-420 grammes were used for these experiments. They were distributed randomly to rule out any influence of sex (Nos 4, 5, 6, 19, 25, 26, 29, Table 1).

Experimental procedures

The thoracic duct was ligated according to the methods described by *Saldeen et al.* (4) and *Azargoschasb* (5): The manubrium sterni was split up to the sternal angle by a short mid-line incision. The left part of the manubrium was retracted to one side and the left sternohyoid muscle was dissected. The whitish distended thoracic duct was then exposed and double ligated (No 1, Table 1). The surgical procedure applied to the sham-operated ani-

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Table 1 Synopsis of the material and methods

	Ligature of the thoracic duct	1	Sham operation	2	Fasting rats	3
postoperative days	1 2 3 5 8 9 10 12 15 90 109 210 360	4	1 2 5 8 15	5	1 2 3 4 5 6 7 8 9 10	6
number of rats	6 8 6 6 3 3 2 5 5 8 2 3 3		<u>3</u>		<u>3</u>	
body weight	taken from all animals	8	idem		idem	9
complete autopsy	done upon all animals	11	idem	12	idem	13
wet weight (liver)	taken from all animals	15	idem	16	idem	17
dry weight (liver)	1 2 3 8 10 12 15 90 109 3 3 1 3 2 2 2 3 2	19	∅		∅	
complete histology	done upon all animals	21	idem	22	idem	23
serum enzymes	GOT GPT LDH A.P.	25	GOT GPT LDH	26		
postoperative days	1 2 4 6 8 10 12 15 20 30 60 90		1 2 5		∅	
number of rats	6 6 6 6 6 6 8 6 6 6 6 6		<u>3</u>			
lipid fractions	total lipids, total cholesterol, triglycerides	29			∅	
postoperative days	1 2 4 6 8 10 12 15 20 30 60 90	∅			∅	
number of rats	6 6 6 6 6 6 6 3 6 6 6 6					
lipid content (liver)	+	32	∅		∅	
postoperative days	1 2 3 8 10 15 90 109		∅		∅	
number of rats	3 3 1 3 2 2 3 2		∅		∅	

mals was exactly the same as the one described above, however, with the exception of the ligature of the thoracic duct (No 2, Table 1). The rats of these two groups were operated upon between 7-9 p.m. and they were given the standard diet Altromin® and water ad libitum.

A control group of fasting rats was given water ad libitum but no food. All animals were kept at room temperature and at a humidity of 50-60% and were housed in cages in groups of 2 or 3 animals.

Analytical procedures

The post-operative weight of the animals was checked every day by means of an analytical balance (Mettler P 1210) (No. 7, Table 1). All animals were killed between 7-9 a.m. either by exsanguination or by decapitation during ether anaesthesia. Then all animals were subjected to a complete autopsy (Nos 11, 12, 13, Table 1).

The wet weight of liver was ascertained by an analytical balance (Mettler P 1210) (Nos. 14, 15, 16, 17, Table 1). Moreover, small liver samples were evacuated at a negative pressure of 5-6 torr for 6 weeks in the presence of phosphorous pentoxide (Nos 18, 19, Table 1). Then the dry weight of liver was determined in the same way as described above.

All organs were fixed in neutral formalin, embedded in paraplast cut into 5 μ sections and

stained for histological studies by one of the following methods: hematoxylin-eosine, Gomori's silver impregnation, Goldner's trichrome stain, hematoxylin-sudan-III method and PAS-reaction according to *Hotchkiss* and *McManus*. All organs were examined by light microscopy. The histological examination of the liver was carried out by each author independently (Nos. 20, 21, 22, 23, Table 1).

The activities of the serum enzymes GOT, GPT, LDH, AP as well as the total lipids, total cholesterol and the triglycerides were measured photometrically by means of suitable reagents (Boehringer, Mannheim) (Nos. 24, 25, 26, Table 1 and Nos. 28, 29, Table 1). 10 healthy rats served as control animals for the normal values of GOT, GPT, LDH, AP; 20 healthy rats were used to ascertain the normal values of the total lipids, total cholesterol and the triglycerides. (These rats are not listed in Table 1).

The lipid concentration of the dry weight of liver was determined according to *Richterich* and *Lauber* (6) after the liver samples had been extracted by methanol/ethanol 2/1 (Nos. 31, 32, Table 1).

Statistical procedures

50 healthy rats were used to evaluate the relationship between the wet weight of liver and the body weight. This relationship is given by the regression line $y = 0,0887 \cdot X^{0,9379}$ (see *Linzbach* (7)). In the case of the rats with ligation of the thoracic duct it was tried to find out in which way the body weight and the time influence the wet weight of liver; for this purpose, the body weight and the time were regarded as independent random variables and the wet weight of liver as a dependent random variable (see *Linzbach* (7)).

Results

General remarks

At the autopsy, the rats with a successful ligation of the thoracic duct show a loss in body weight; the same applies to the sham-operated rats. They regain their original weight around the 9th-14th day. During the 1st-15th day we find a granulation tissue, however, no morphological signs of infection. There is a chylous fluid in the pleural and abdominal cavities. The thoracic duct is whitish and distended and the same applies to the mesenteric lymphatics. In the long-term experiments the animals exhibit no obvious alterations. As regards the morphological changes in the lungs, hearts and kidneys see *Cremer* et al. (8, 9, 10). At the autopsy of the sham-operated rats we find a granulation tissue at the site of operation during the 1st-15th day, but no marked changes of the other organs whatsoever. The autopsy of the fasting rats discloses signs of starvation, e.g. a loss of adipose tissue and brown atrophy of the inner organs.

Statistical results

The wet weight of liver of almost all rats with successful ligation of the thoracic duct is considerably reduced. This becomes obvious in Fig. 1 in which the wet weights of liver of the rats with ligation of the thoracic duct and those of healthy control animals are charted. Furthermore, this figure shows that there are no differences between both sexes. Fig. 1b shows the same facts in a way which makes them comparable to the dry weights of liver. In earlier studies on lungs, hearts and kidneys (8, 9, 10) we undertook a planimetric determination of the surface area of the perivascular spaces (A) and of the vascular lumen (I);

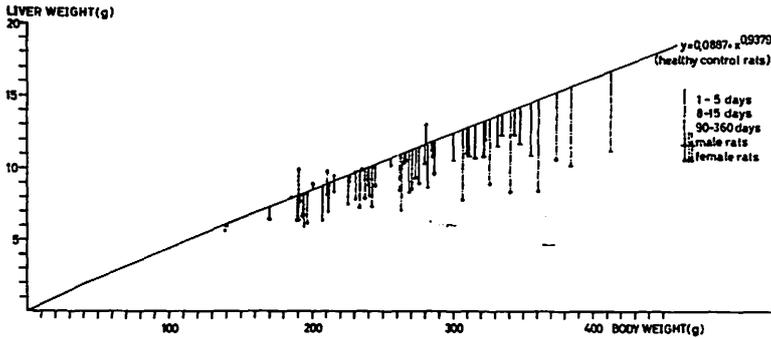


Fig. 1a Wet of weight liver of rats with ligature of thoracic duct plotted versus those of healthy control rats as a function of time.

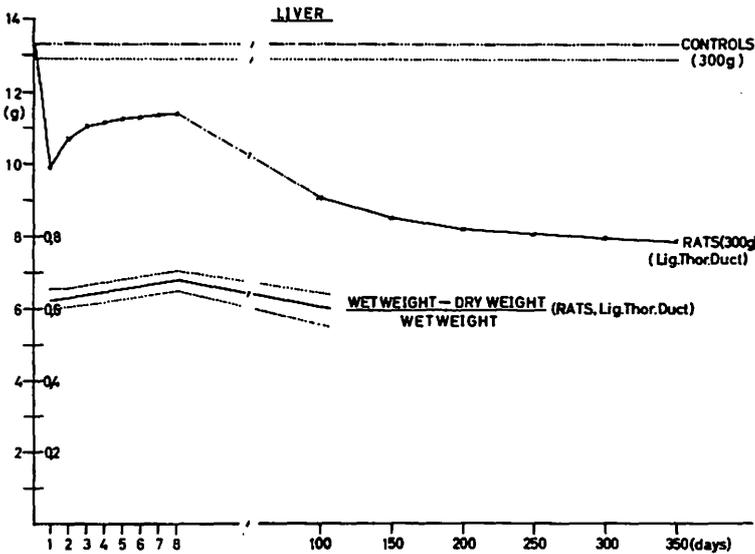


Fig. 1b Relationship between wet weight of liver, body weight of healthy control rats (top) and rats with ligature of thoracic duct (center) and time. The relationship is given by the regression line $y = 10^{(a + b_1 \cdot \lg x_1 + b_2 \cdot \bar{x}_2)}$. y stands for wet weight of liver, x_1 for body weight, x_2 for time.

1st - 8th day:	a = -0,6067		
	$b_1 = 0,4845 < 0,6750 < 0,8653$		
	$b_2 = -0,1261 < -0,0693 < 0,0124$		
10th-90th day:	a = -1,1567		
	$b_1 = 0,2772 < 0,8351 < 1,3931$		
	$b_2 = -1,44 < 1,1020 < 3,644$		
90th-360th day:	a = -0,4082		
	$b_1 = 0,2768 < 0,5165 < 0,7561$		
	$b_2 = 3,0773 < 8,5663 < 14,0553$		

Relationship between the dry weights of liver and the wet weights of liver of rats with ligature of the thoracic duct as a function of time (bottom). The relationship is given by the regression line: $y = a + b \cdot x$. Y stands for $\frac{\text{wet weight of liver} - \text{dry weight of liver}}{\text{weight of wet liver}}$; x stands for time.

1st - 12th day:	a = 0.06183	b = 0,0025 < 0,0076 < 0,0126
15th-109th day:	a = 0.7382	b = -0,00216 < -0,00127 < -0,00038

($\frac{A}{A+I}$). Since, for anatomical reasons, such a procedure cannot be applied to the liver — the lymphatic vessels within the portal tract are not constructed as perivascular spaces — we choose an alternative formula to give comparable results. As can be concluded from this figure, the small increase in the wet weight of liver results from an accumulation of fluid during the 1st-8th day, i.e. from a lymphoedema.

Histology of the liver of rats with a successful ligation of the thoracic duct
(Nos 20, 21, Table 1).

After 24 hours, the spaces of Disse are slightly dilated and the endothelial cells have raised from the hepatocytes. The spaces of Disse in the central areas of the liver lobule contain a less dense fluid which takes on a lighter colour when stained with eosine than the — denser — fluid in the sinusoids and the spaces of Disse at the periphery of the liver lobule. After 4-6 days, the cytoplasm of the hepatocytes shows vacuoles while still staining quite normal. After 6-8 days, the vacuolisation is accompanied by a considerable loss of basophilia of the cytoplasm. In several liver lobules isolated cell necroses can be detected, mainly at the periphery of the lobule. Between the 6th-8th day the walls of the Vv. centrales are severed from the bordering liver parenchyma by a band of oedema (Fig. 2). A few days later, dilated lymphatic vessels are found in the periadventitial connective tissue of the Vv. hepaticae. After a longer period, i.e. after 210-360 days, there is a reduction of the nuclear and cell volume of the hepatocytes and an accumulation of lipofuscin within their cytoplasm.

After the 2nd - 3rd day the portal tracts lose their characteristic form and change into round fields. The spaces of Mall are filled with an eosinophilic fluid and several smaller ectatic lymph capillaries are found grouped around the bile ducts whose epithelia show

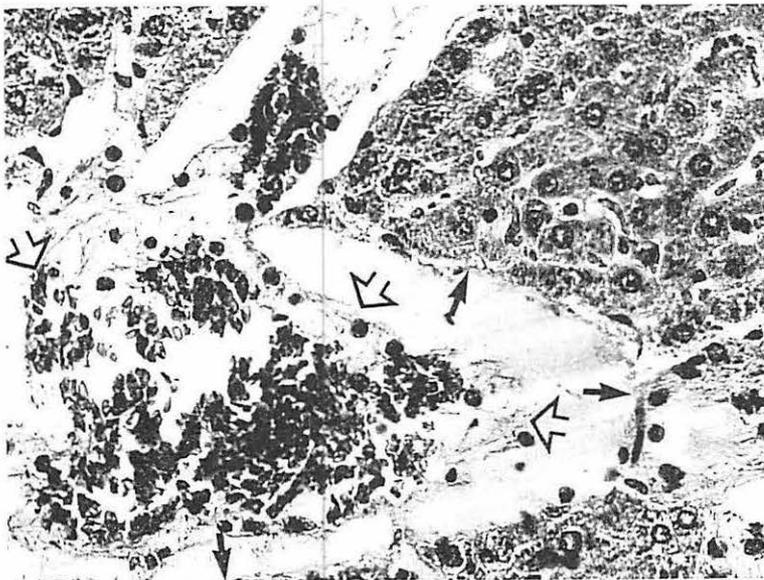


Fig. 2 Liver of a rat, 8th post-operative day. V. sublobularis. Extensively dilated periadventitial lymph clefts surrounding the hepatic vein. The light arrows point to the vein wall and the dark arrows to the border of the parenchyma. H.E. stain, x 120.

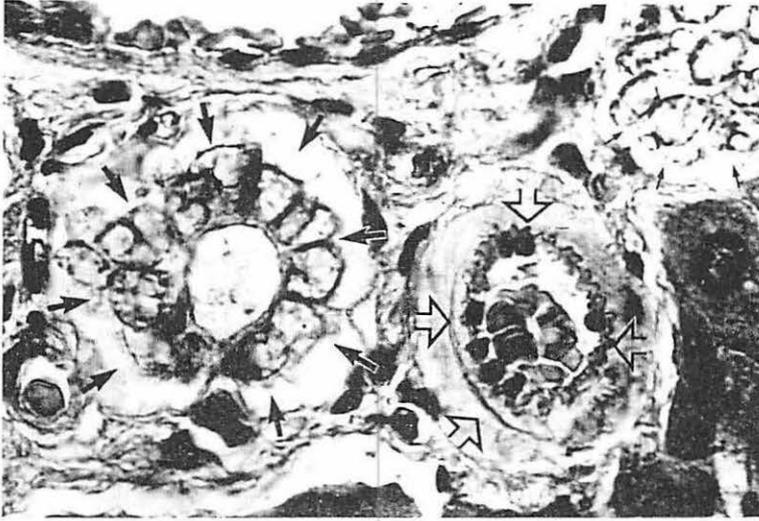


Fig. 3 Liver of a rat, 2nd-3rd post-operative day, portal tract. The branch of the A. hepatica within the portal tract exhibits a thickened wall of a glassy appearance (light arrows). The epithelia of the bile duct show large retronuclear vacuoles (dark arrows). H.E. stain, x 320

large retronuclear vacuoles (Fig. 3). Between the epithelia of the small bile ducts of Hering an interstitial fluid entering the lumina can be observed (Fig. 4). Around the 6th-8th day a pale eosinophilic fluid is noticed within the bile ducts. This fluid takes on the same colour as the fluid in the surrounding lymph capillaries. This is suggestive of lympho-biliary

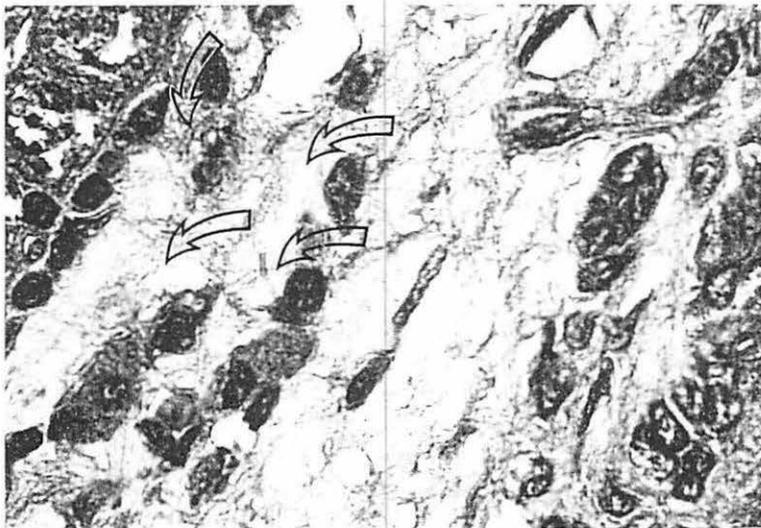


Fig. 4 Liver of a rat, 4th post-operative day, portal tract. Marked oedema of the portal tract, entering a bile duct of Hering between the hepatocytes (light arrows), bile duct epithelia on the left side assuming dark colour. H.E. stain, x 320

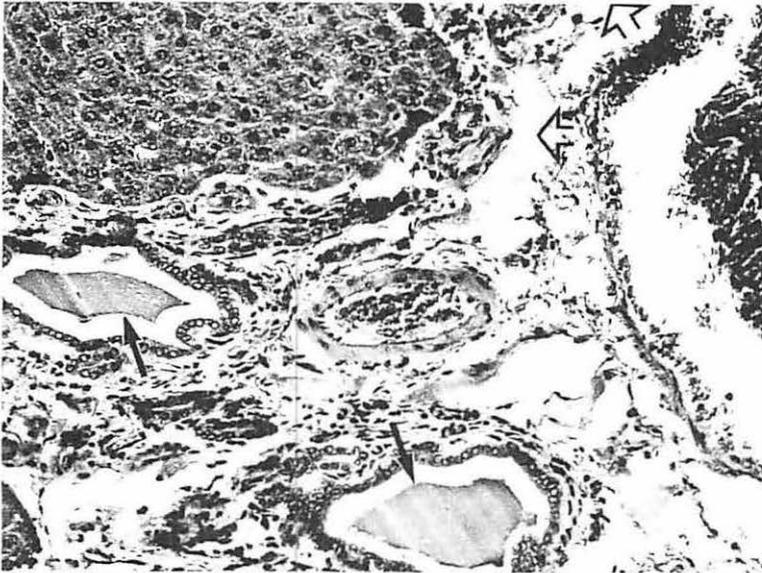


Fig. 5 Liver of a rat, 8th post-operative day, section of a portal tract. In the dilated bile ducts (dark arrows) an optically denser fluid can be seen, which assumes colour in a manner similar to that of the fluid in the dilated lymph vessels. Dilated spaces of Mall (light arrows). Numerous lymphangiectases can also be detected. H.E. stain, x 128

fistulae (Fig. 5). No lymphatico-venous communications can be detected. The branches of the A. hepatica within the portal tracts exhibit a thickened wall of a glassy appearance. A larger magnification shows that this is due to an oedema of the smooth muscle cells. No oedema of the intima can be detected (Fig. 3). In the case of animals which were observed for a longer period a discrete increase in concentrically layered connective tissue around the bile ducts and an occasional fibrosis of the interlobular and intralobular tissue is observed.

The histological examination of the sham-operated rats (No 22, Table 1) and the fasting rats (No. 23, Table 23) reveals no pronounced alterations compared to healthy test animals. In the case of the fasting rats, the histology of the liver is of some interest: On the 1st day a drastic reduction of the cytoplasmic glycogen is noticed which corresponds to a reduction of the cell volume of the hepatocytes. On the 5th fast-day cell necroses and cytoplasmic debris are observed in the central parts of the liver lobules. These necrotic materials are phagocytized by Kupffer cells. No signs of a lymphoedema can be detected.

Serum enzymes of the rats with ligation of the thoracic duct and of sham-operated rats

(Nos 24, 25, Table 1).

During the first day the serum enzyme activities show a marked elevation of LDH as well as a fairly small elevation of the GOT and GPT values. Later on, the LDH values remain slightly elevated whereas GOT shows small oscillations around the normal values. In the case of GPT, there are no significant deviations from the normal values. The alkaline phosphatase shows a drop during the 1st day and remains diminished during the whole experiment (Fig. 6a).

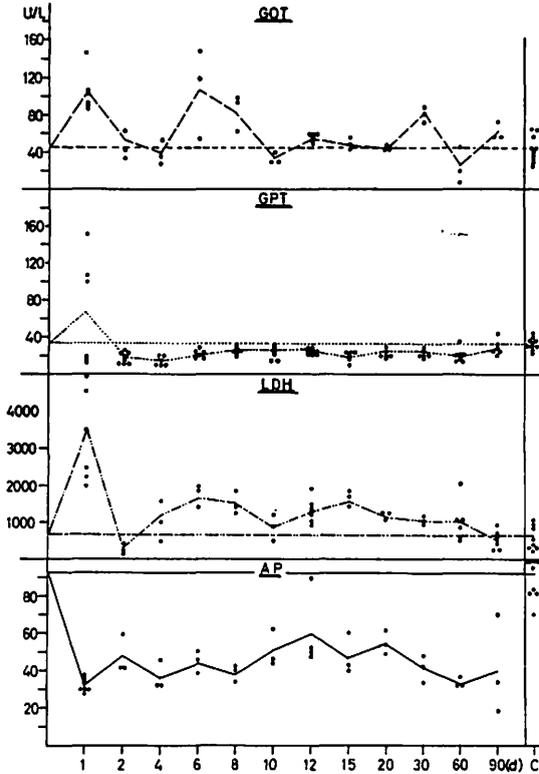


Fig. 6a Serum enzyme activities in rats with ligation of the thoracic duct as a function of time. The results are expressed as U/L.

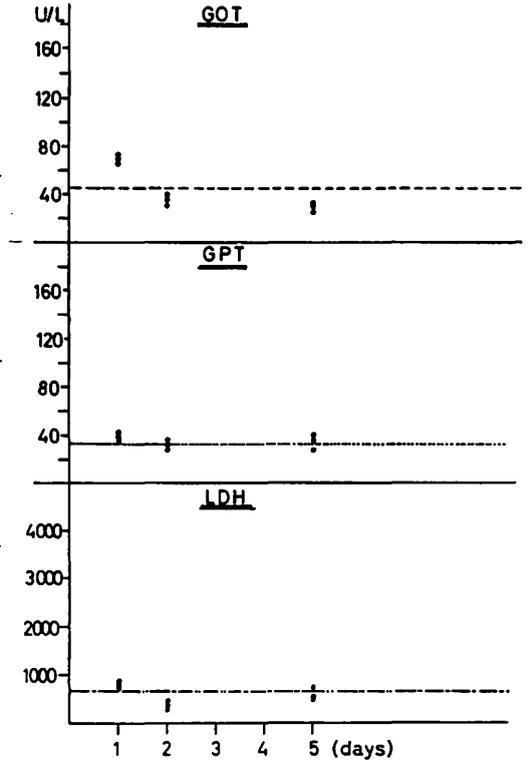


Fig. 6b Serum enzyme activities in sham-operated rats as a function of time. The results are expressed as U/L.

The serum enzymes of the sham-operated rats (No 26, Table 1) show only slight elevations on the first two post-operative days. Thereafter, no further changes in the enzymes, activity can be observed (Fig. 6b).

The lipids of rats with ligation of the thoracic duct

During the whole experiment the total lipids show slight oscillations around the normal values whereas the total cholesterol remains slightly but insignificantly elevated. The triglycerides however, show a marked biphasic decline with the first lowest value on the 1st post-operative day and the second lowest value around the 15th post-operative day. The normal values regained around the 20th day (Fig. 6c).

The lipid concentration of the dry weight of liver of rats with ligation of the thoracic duct (No. 32, Table 1) declines during the first 10 post-operative days and then increases again but does not regain the initial content by the 109th day (Fig. 7).

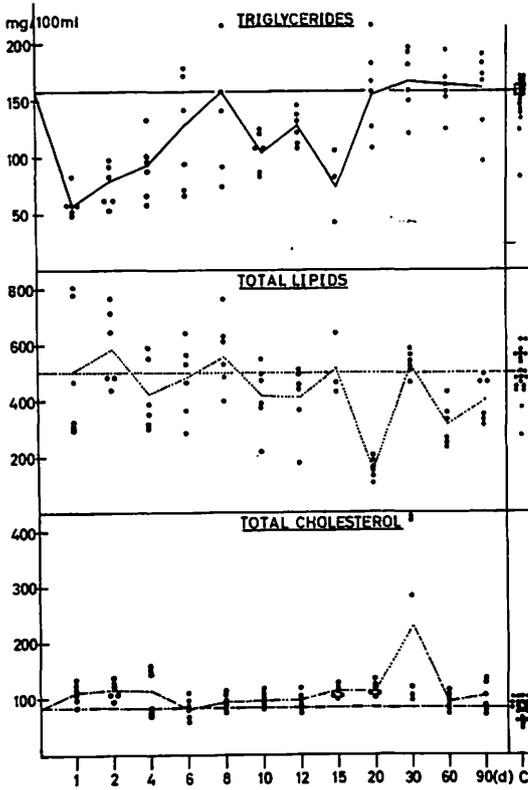


Fig. 6c Lipids of the serum of rats with ligation of the thoracic duct as a function of time. The results are expressed as mg/100 ml. C stands for controls; the horizontal lines stand for normal values.

Discussion

We should like to start with a critical appraisal of the method applied. The blockage of the thoracic duct before its entry into the left angulus venosus at the base of the neck results in a generalized lymphoedema (with the exception of the right side of the head). This means that the biochemical and histological alterations described above are to be examined as to the extent to which these alterations are attributable to a lymphoedema

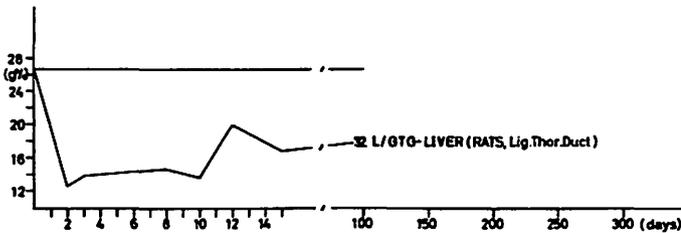


Fig. 7 Lipid concentration of livers of rats with ligation of the thoracic duct as a function of time. The results are expressed as percentage by weight based on the weight of dry tissue.

within the liver or to which they are secondary manifestations of alterations of other organs, e.g. the heart, kidneys etc.. This question can only be answered by experiments in which the lymphatic vessels within the liver hilum are selectively obstructed. These experiments were carried out by *Huth* et al. (2, 3) and *Gerlach* et al. (11). The light- and electron-microscopical observations made by *Huth* et al. (2, 3) and the biochemical findings of *Gerlach* et al. (11) are similar to ours. This supports our view that our findings are mainly attributable to a lymphoedema in the liver and only very little to the influence exercised by other organs.

After this critical appraisal we should like to point out the following histological findings:

- 1) Despite a marked oedema of the wall of the A. hepatica, this lesion does not develop into a manifest atherosclerosis (12, 13) presumably because the lymphoedema does not result in fragmentations of connective tissue fibres (14);
- 2) there are histological findings which are indicative of lympho-biliary fistulae. This fact might explain why even in transplanted livers no complete lymphoedema can be obtained;
- 3) after 16 days almost all morphological signs of a lymphoedema within the liver have disappeared. This is probably due to the formation of collaterals as can be concluded from the extensive studies made by *Akisada* et al. (15) and *Malek* (16);
- 4) there are histological and biometrical signs of an atrophy of the liver. However, the details of the basic mechanisms leading to the atrophy are not known to us. Since the fibrosis of the interlobular and intralobular tissue is only discrete and no marked necrosis of the hepatocytes can be detected in the animals which were observed during a long period, these changes cannot account for the atrophy of the liver (and of the heart as well as of the kidneys, 9, 10).

Blalock et al. (17) tried to produce a complete lymphatic blockage in 52 dogs and 22 cats. Operations were carried out to block the lymph ducts in the neck and chest, to destroy the cisterna chyli and to interfere with the drainage of the mesenteric lymphatics. Assessed by the cells in the blood, a temporary obstruction was obtained in many of the animals, but soon the bloodcell picture returned to normal. Permanent complete blockage was, however, obtained in 3 dogs in whose blood the lymphocytes and eosinophils almost disappeared. The animals lost weight rapidly and were killed when it was obvious that they were going to die.

We observed similar post-operative courses in our series. It therefore could be that a permanent complete blockage results in the death of the animals, whereas an impairment of the lymphcirculation results in the atrophy of the organs.

Yoffey and *Courtice* (18) therefore suppose that an unimpaired circulation of lymph is essential for the life of the individual.

Special attention should be paid to the alkaline phosphatase. The alkaline phosphatase content of the intestinal lymph is higher than that of plasma. When all intestinal lymph is drained from the body, the concentration of the alkaline phosphatase in the plasma is greatly reduced. This suggests that the serum alkaline phosphatase comes to a large extent from the intestine and is transported to the bloodstream mainly by the lymphatics (19). So our findings of a decreased content of alkaline phosphatase in the plasma after blockage of the thoracic duct are in keeping with the statement made by *Courtice* (19). The same applies to the findings in respect of the lipids. The lipids in the plasma consist of triglycerides, phospholipids and cholesterol esters including small amounts

of free fatty acids and free cholesterol. These lipids are also in the lymph coming from all tissues of the body, i.e. the lipids in the lymph are derived from the circulating plasma by way of extravascular circulation (18). It could therefore be expected that the blockage of the thoracic duct would result in an interruption of 70% of this lipid stream (19); (the remainder is carried in other final lymphatic pathways: cervical, subclavian and right lymph ducts). This is particular true of the triglycerides. As can be seen from Fig. 6c, the triglycerides show a decrease during the first 15 days. This drop must be examined as to the question of 1) an altered invasion of the triglycerides into the plasma (decreased chylomicron-synthesis by the mucosa cells, interruption of the extravascular circulation, blockage of the thoracic duct, diminished capacity of the liver to synthesize triglycerides from the FFA of the adipose tissue or a decreased mobilisation of FFA from the adipose tissue); 2) an altered elimination of the triglycerides from the plasma (an increased function of the lipoprotein-lipase, an increased utilisation by the muscle cells and other tissues); 3) an alteration of the volume of distribution.

With respect to our findings of a decreased level of plasma in the alkaline phosphatase, we are of the opinion that this decrease in triglycerides is induced by the blockage of the lymphatic circulation. After 15 days the values of the triglycerides return to normal, a date which is in good conformity with our histological findings and the literature concerning the restoration of the lymphatic vessels after experimental destruction (16).

As can furthermore be seen from Fig. 6c, the basic pattern of the total lipids and total cholesterol are not significantly affected by our surgical procedure.

Finally, the question of the decrease in the lipid concentration of the dry weight of liver is to be discussed. According to *Engelhardt* (20), the liver stores triglycerides in proportion to their plasma levels. Since the plasma levels are reduced, our findings of a decreased lipid content of the dry weight of liver are in line with the statement made by *Engelhardt* (20).

This experimental model might serve to emphasize the importance of the lymphatic vessels of the liver in the fields of human medicine and pathology, especially in cases of liver transplantation and carcinomatous lymphangiosis.

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Value of Lymphography in Detecting Metastatic Cloacogenic Carcinoma

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Summary

Cloacogenic Carcinoma is a rare and highly malignant tumor arises from the transitional cloacogenic zone of anorectal junction. These tumors metastasise by direct invasion and via the lymphatic channels to the regional lymph nodes. Lymphography was utilized to diagnose metastatic cloacogenic carcinomas and in 3 patients positive nodes were found on lymphography. This is a preliminary report describing the findings of metastatic cloacogenic tumors on lymphography and the author recommends utilization of this diagnostic procedure in the work up of cloacogenic carcinoma.

Value of lymphography in detecting metastatic cloacogenic carcinoma

Cloacogenic carcinoma is a highly malignant lesion arising from the transitional cloacogenic zone of anorectal junction. Although it has been recognised as a distinct clinico-pathologic entity since 1956, information regarding radiological diagnosis still is