SURGICAL JAUNDICE:

AN EXPERIMENTAL STUDY

THESIS SUBMITTED FOR THE DEGREE OF

Ch.M.

UNIVERSITY OF EDINBURGH

BY

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APRIL, ~~1957~~ 1959.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to:-

The Mayo Foundation for Medical Research who generously made this study possible;

Dr J.L. Bollman and Dr J.H. Grindlay for their help and criticism;

Dr F.C. Mann and Dr C.F. Code for their advice and encouragement;

Dr E.V. Flock for her advice and guidance with the chemical techniques;

Dr H.N. Hoffman II who kindly performed the chromatographic analyses;

Dr J. Berkson and Dr R.A. Robb for advice on statistical matters;

Mr Emery van Hook, chief technician to Dr Bollman, who performed the liver lymphatic canulations in rats.

CONTENTS

INTRODUCTION AND DEFINITION OF TERMS

Classifications of jaundice abound in medical literature. Some have served a useful purpose (McNee, 1922, 1923; Rich, 1930), others have not. The current tendency to subdivide jaundice into 'Medical' and 'Surgical' groups has, at least, the virtue of simplicity. For the purpose of this thesis the term 'Surgical Jaundice' will be used to refer to that variety which follows complete mechanical obstruction of the common bile duct and it is with the pathogenesis of this type that the present study is concerned.

More than 150 years have passed since Saunders (1803) ligated the common bile duct in a dog. His observation that bile pigment promptly accumulated in the lymph passing through the thoracic duct caused considerable interest. Since then a great deal of experimental work has been done and much has been written on this phenomenon, but opinion is still divided as to its significance.

It has been claimed that this pigmentation of the lymph may be due to 'regurgitation' of formed bile from some proximal part of the biliary tree into adjacent lymph spaces in the liver, from which it would pass to the thoracic duct and thence to the blood itself, and that such is the mechanism /

mechanism of 'Surgical Jaundice' (Bloom, 1923; Gonzalez-Oddone, 1946; Heidenhain, 1868; Watson, 1940). Scrutiny of the existing evidence for this belief shows it to be based mainly on similar observations of the increase in the bilirubin content of thoracic duct lymph after biliary obstruction. These observations were made during acute experiments on anaesthetised dogs from which the gall bladder had been removed. Many authorities have not been completely satisfied with this explanation and several alternative theories have been advanced (Mayo and Greene, 1929; Mendel and Underhill, 1905; Rich, 1930; Wertheimer and Lepage, 1898; Whipple and King, 1911). These will be reviewed below.

It is in the minute anatomy of the liver structure that the primary gaps in our knowledge exist. Much has been learned in the past 30 years about the intra-hepatic vascular pathways (Andrews, 1953a, b, 1955; Andrews et al. 1951, 1953; Bauer et al., 1932; Bearn et al., 1951; Chakravarti and Tripod, 1940; Grayson and Johnson, 1953; Watkin and Mann, 1942), but our knowledge of the disposition of tissue spaces and of the lymphatic bed is scanty. It seems clear that large lymphatics run in the connective tissue of the portal triad (Bollman, 1950; Popper, 1955) and /

and also that some lie within the walls of the larger biliary ducts (Johnson and Mann, 1950). Where the intra-lobular tissue spaces are situated in unknown. Indeed, it has been claimed that they do not exist (Browicz, 1900). But this, on general grounds, seems unlikely.

The existence of the so-called spaces of Disse (Disse, 1890; MacGillivray, 1865) and Mall (1901 and 1906) has also been the subject of much dispute (Patek, 1945) but the former, at least, has now been fairly widely accepted (Flock et al., 1947; Hanger, 1950; Knisely et al., 1948; Pavel, 1949; Rouiller, 1954; Wintrobe et al., 1954). Neither has been shown to bear direct anatomical relationship to the biliary canaliculi or to the canals of Hering; except in schematic drawings (Rich, 1930).

In addition, there is as yet no clear idea of what constitutes the proximal part of the biliary tree (Andrews, 1955). That a canalicular structure exists is certain. Its connection with the biliary tract, thought to be at the level of the finest cholangioles, has not been conclusively demonstrated (Andrews, 1955; von Kölliker, 1852). Many authorities are satisfied that the canals of Hering complete this connection (Caroli, P., Personal Communication; Knisely, 1952; Maximow and Bloom, 1952; McIndoe, 1928), some are not (Maegraith, Personal Communication; Bollman, J.L., Personal /

Personal Communication; Andrews, 1955). In his "Handbook of Microscopic Anatomy" (1852) Professor von Kölliker had this to say, "Often as I have sought for a direct connection of the finest canals with the hepatic networks, I have not directly observed it. Which is, indeed, by no means sur-:prising if we consider the softness of the parts with which we have to do: but unfortunately the result is a hiatus in the minute anatomy of the parts which can hardly be made good by hypotheses."

It may be that electron microscopy will help to settle this controversy, but so far reports of such work are few and far between. From those available, it appears that the space of Dissé is a definite entity (Rouiller, 1954). Nevertheless, until such time as the anatomical relation-:ships required to satisfy a physiological hypothesis are founded on fact, this concept is confined to the realms of speculation.

Within the last ten years experimental methods have been developed which seem to give promise of further information. In 1945 Dr. F.C. Mann suggested to Dr. J.H. Grindlay that a study of the liver lymph might provide a new approach to problems in hepatic physiology. Grindlay developed a technique whereby it is possible to canulate the /

the main hilar lymphatic trunk as it leaves the dog's liver (see Appendix A) with a polyvinyl tube and so to obtain a liver lymph fistula in the unanaesthetised dog (Grindlay et al., 1948, 1950). Subsequently some results of this work have been reported (Cain, 1947; Cain et al., 1947; Flock, et al., 1947; Nix et al., 1951).

It seemed possible that this technique might provide further information about the pathogenesis of 'Surgical Jaundice', in that the liver lymph itself could be studied rather than that from the thoracic duct. Also it would now be possible to continue to make observations on the unanaesthetised animal for periods of up to four days and, by using techniques of delayed cannulation, to extend the investigation to include the study of chronic biliary obstruction.

Recently outstanding work by Cole, Lathe and Billing (1954) on 'reverse phase' chromatography and on the chemistry of bilirubin has provided another important biochemical tool (Billing and Lathe, 1956). It seemed that chromatographic methods might help to identify the pigments present in the liver lymph during experimental surgical jaundice. Thus further information might be obtained about the source from which they had come.

Finally, it is necessary to recognise that the term 'Regurgitation' /

'Regurgitation', introduced by Rich in 1930, has been and is being used in a number of ambiguous and confusing ways. For some it has meant the passage of bile from the biliary tree into the liver lymph (Gonzalez-Oddone, 1946; Watson, 1940, 1955), for some into the blood (Popper, 1955; Rich, 1930) and for others into both media (Mixer et al., 1947). Etymologically, of course, each of these usages of the word is justifiable, since it simply means 'to vomit back'. All imply that it is formed bile from the biliary tree which suffers this fate. The difference of opinion exists mainly in the matter of its immediate destination. Whether the source of this pigment is, in fact, the biliary tree does not appear to have been seriously questioned since Rich's (1930) paper was published.

It was the aim of this investigation to study the development of 'Surgical Jaundice' in experimental animals. An attempt was therefore made to map out the Standard Response to Biliary Obstruction in terms of the changes it produced in the concentration of bilirubin or injected bromsulphthalein (B.S.P.) in the lymph coming from the liver and in the circulating blood. Once these patterns had been established more intimate studies on the Mechanism of the Standard Response became possible.

GENERAL STATMENT OF THE PROBLEM TO BE INVESTIGATED

What is the nature and sequence of the events which produce the jaundice after complete mechanical obstruction of the common bile duct?

REVIEW OF THE LITERATURE

Introduction

During the last 150 years a great deal of work has been done on the pathogenesis of obstructive jaundice. Three main methods of study have been used; of which the gross experimental approach has been, perhaps, the commonest. These studies on experimental animals with biliary obstruction have been largely confined to measurements of the changes in bilirubin concentration in thoracic duct lymph and venous blood. In a few of them the fate of injected B.S.P. under these conditions has been followed. Another common method of study has been the histological one. In this the microscopic sequelae of biliary obstruction have been examined and attempts have been made to observe directly the processes involved in producing the icterus. A more recent technique employed has been that of fluorescence microscopy, but here, as with both of the previous techniques, differences of opinion exist as to the interpretation of results.

Since the study presented in this thesis is of an experimental nature the literature will be reviewed from that standpoint. It may be divided broadly into two groups; the /

the majority of the workers in this field believe that the source of the pigment responsible for the jaundice which follows biliary obstruction is the biliary tree itself; a much smaller group deny this.

Of those who believe that the source of the pigment is the biliary tree most workers favour the 'regurgitation theory'. But here the agreement ends. A schism exists in opinion as to the immediate destination of the regurgitated substances. Many take the view that they pass directly into the lymph spaces in the liver and are carried thence via the thoracic duct to the blood. Others are convinced that they are routed at once to the blood in the sinusoids, while the remainder believe that both pathways are involved. A few workers however have presented anatomical evidence of direct connections between the bile capillaries and the lymph spaces (Pavel, 1949; Rouiller, 1954), or blood vessels (Nauwerck, 1897; von Haller, 1776) within the liver. They suggest, therefore, that the constituents of the bile pass directly from the biliary tree and are not regurgitated.

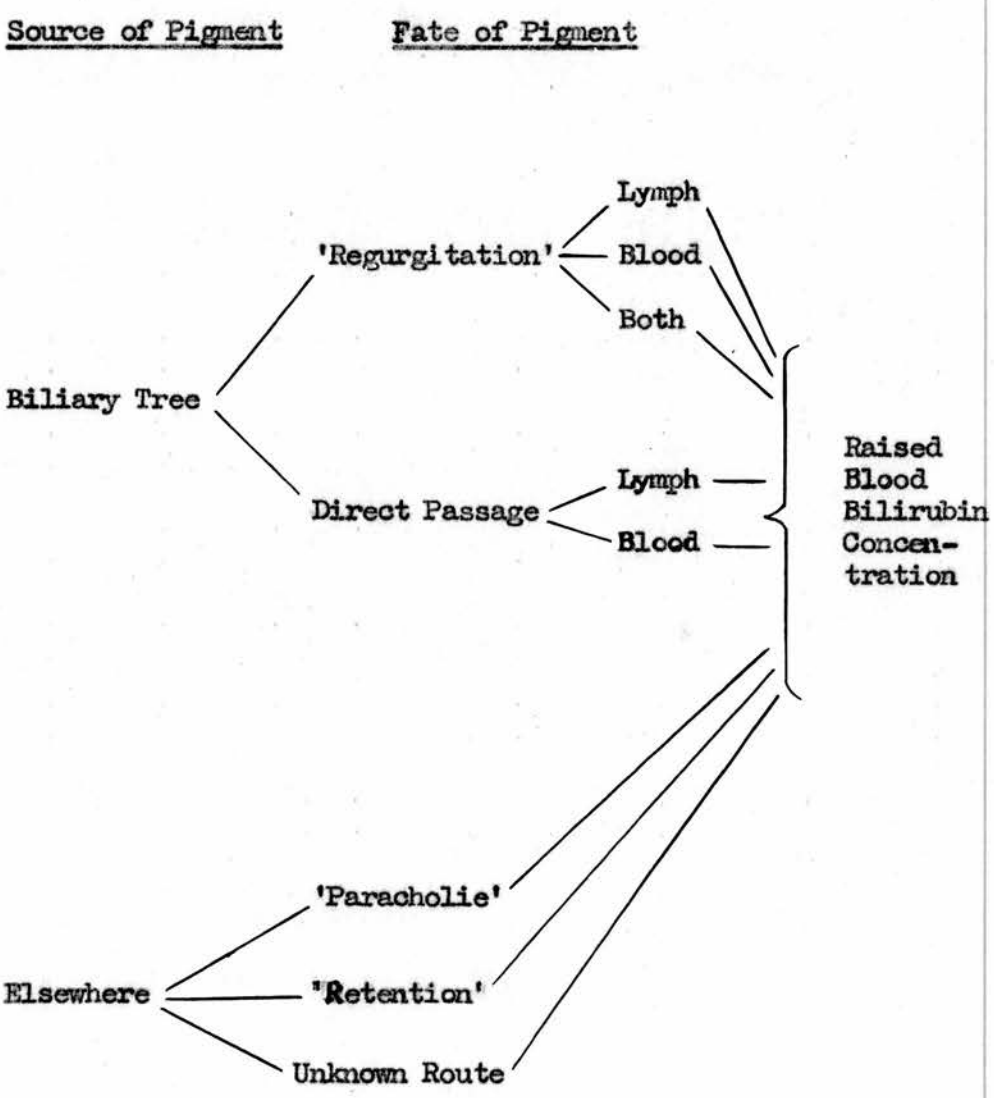
No general agreement exists amongst that small group of dissenters who do not accept the premise that the biliary tree is the source of the pigment. Some of these workers (Minkowski, /

(Minkowski, 1892; Sterling, 1911) believe that a reversal of polarity occurs in secretion from the hepatic cell (paracholie) and that the pigment is returned forthwith to the lymph and blood, and some that there may be simple retention of pigment perhaps with 'active secretion' from the blood into the lymph for a time (With, 1947). Those who remain are uncommitted (Klatskin, 1948-1949; Shafiroff, et al., 1939).

A synopsis of the above theories is given on p. 11.

THE PATHOGENESIS OF THE JAUNDICE IN BILIARY OBSTRUCTION

SYNOPSIS OF THEORIES ADVANCED IN THE LITERATURE



Published work on the pathogenesis of obstructive jaundice is first of all reviewed under the headings of the various theories which have been summarised above. This includes the pertinent histological studies. The excerpts are presented wherever possible in chronological order.

The Regurgitation Theory

It will be seen from the synopsis on page 11 that a difference of opinion exists amongst those who ascribe to this theory as to the route taken by the pigment after it has been regurgitated from the biliary tree. Some believe that it passes into the lymph, others that it enters the blood and the remainder that it may pass by both routes. The relevant literature will be summarised, under these headings, in that order.

Regurgitation into the Lymph

Scrutiny of the existing literature and opinion on the pathogenesis of the jaundice in biliary obstruction has shown that, to date, more authorities incline to this view than to all other alternative theories put together.

In was in 1795 that the first ligation of the common bile duct in a living dog was carried out. This was done by /

by Saunders (1803) and the animal was sacrificed two hours later. At post-mortem he found the liver lymphatics and the thoracic duct distended with bilious fluid, and also that the serum of blood drawn from the hepatic vein appeared to be more yellow in colour than that from the jugular vein.

In 1868 Heidenhain attempted to determine the site of escape of bile from the biliary tree into the lymph during biliary obstruction. He injected a solution of sodium indigo-sulphate into the bile ducts and found that the skin, mucous membranes and urine rapidly became blue. Histological examination of the liver showed that there was no colouration within the lobules themselves, the blue colour being confined to the interlobular tissues. It was not seen in the bile capillaries. He concluded that during biliary obstruction bile escapes from the interlobular bile ducts and passes into the tissue fluid from which it is carried away by the lymphatics. He claimed that after ligation of the common bile duct bile pigments and bile salts accumulated in large amounts in the thoracic duct lymph and that the blood serum contained no trace of either.

Fleischl (1874) ligated the common bile duct of dogs and collected the lymph from thoracic duct fistulae. He found bilirubin /

bilirubin to be present in large concentrations in the lymph while, up to five hours, he could detect none in the blood-stream. Bile acids behaved in a similar manner.

In 1875 Kunkel claimed to have crystallised bile salts from the liver lymph during biliary obstruction. It seems that he succeeded in isolating 872 mg. of bile salts from 200 ml. of lymph!

Kufferath (1880) claimed to have detected bile and bile salts in thoracic duct lymph but not in the blood and reached the same conclusion as Fleischl. Both used the Gmelin(Tiedemann and Gmelin, 1827) and Pettenkofer test.

Harley (1893) ligated the common bile duct in two dogs and in one of these he also tied off the thoracic duct. In this paper, in 1893, he observed that in the dog with the patent thoracic duct bile appeared in the urine after a few hours of biliary obstruction, while in the other dog it could not be identified until eight days had passed. In a second series of 18 canine experiments he ligated the common bile duct and the thoracic duct in one group and in the remainder the common bile duct only. A few days later he tied the thoracic duct in the second group of dogs. He reported that 11 of these experiments gave evidence that the bile was carried to the blood 'solely by the lymphatic vessels'. /

vessels'. He explained the presence of bile pigment in the urine of the other seven preparations by the fact that it must have passed through new lymphatic pathways which had opened up alongside the old ones. It appears from his data however, that in five of the experiments in which the bile ducts had been tied some days before the thoracic duct was ligated, the urine continued to contain bile on each day without exception.

In 1897 Meltzer repeated experiments carried out by Starling and Tubby (1894) which will be described below, page 29 . He injected indigo-carmine and methyl blue into the pleural and peritoneal cavities of animals and found that the colour appeared in the thoracic duct lymph before it was seen in the urine.

In 1902, 1903, 1908 and 1920 Eppinger carried out the first important studies on the histological changes in the bile capillaries during obstruction of the common bile duct. He claimed to have shown that the cannaliculi in patients with long-standing jaundice were greatly distended, tortuous and that ruptures had occurred into the adjoining lymphatic spaces. He could find no evidence that rupture of the bile capillaries allowed the escape of bile into the hepatic sinusoids and believed that it passed into the perisinusoidal space from where it was carried by the liver lymphatics /

lymphatics to the thoracic duct. It was only after 24 hours of obstruction that he was able to demonstrate an increase in the bilirubin content of the blood.

In 1904 Abramov and Samoilowicz (Brauer et al., 1950) and in 1905 Abramov confirmed Eppinger's findings. Jagic (1903) however, ligated the common bile duct in a series of canine experiments and found that there was no histological evidence of rupture of the bile canaliculi up to the fifth or sixth day. His findings were confirmed by Ogata (1913) in an extensive series of experiments performed on many animals including the dog. He found that dilatation of the bile capillaries followed obstruction, but that rupture occurred only in the late stages. He believed that a necrosis of liver cells preceded rupture of the canaliculi during biliary obstruction and presented as evidence the fact that jaundice was established long before rupture could be seen. In 1925 Kodama and Hiyeda (1925), working independently, were also unable to find evidence of rupture of the bile capillaries in early obstructive jaundice and claimed to have confirmed Ogata's findings. It was in 1932 that Cameron and Oakley published their classical paper on the histological sequelae of biliary obstruction. It contains an exhaustive review of the subject. In their experiments /

experiments, which were carried out on rats, they detected bile duct hyperplasia and cholangiectasis but found little evidence of rupture. In 1939 Roholm and in 1948 Popper finally demonstrated that rupture was due to agonal change.

To resume the review of the gross experimental studies, Gerhardt (1897, 1905) in 1905 injected sodium indigo-sulphate into the bile ducts, using Heidenhain's technique, and confirmed his findings.

In 1905 Rolleston, in his textbook, took the view that the main pathway of bile from the liver after obstruction was by way of the lymphatics.

Herring and Simpson (1907) using a technique similar to that of Heidenhain (1868), injected carmine gelatin at various pressures, into the bile passages of various animals. In several experiments, including some on the cat and dog, carmine gelatin was found in the lymph trunks issuing from the liver and in the lymph glands in relation to the portal vein. They now attempted to find the time at which bile appeared in these lymph vessels after obstruction of the common bile duct. After clamping the cystic and common bile ducts in a cat they severed a lymph vessel on the portal vein, absorbing specimens of the lymph, every two minutes on pieces of blotting paper. They found that these became heavily /

heavily stained with bile one hour later.

Adami (1908), in his textbook, stated, 'Clearly, the normal path by which the bile reaches the circulation is by way of the lymphatics'. He quoted Harley's (1893) experiments in support of this view.

Similarly in their textbook, 1909, Kelly, Osler and McCrae affirmed that it has been 'repeatedly demonstrated' that the bile travels by the lymphatic pathways under these circumstances.

Bloom, in his studies in 1923, had the advantage that he could use the van den Bergh (1918a, b, 1913) test to establish the presence of bilirubin. He pointed out that studies carried out before his time were open to criticism on the grounds of the chemical techniques used. Hitherto the Gmelin or Salkowski tests were employed. He stated that the Gmelin test is sensitive only to a dilution of 1 in 80,000 or more and is liable to fail if it is used on solutions containing protein. Moreover, the Salkowski test will not demonstrate successfully the presence of bilirubin in low concentrations unless large quantities of serum are available. The van den Bergh test (Andrews, 1924; Lephene, 1921; Rosenthal and Holzer, 1921; van den Bergh, 1918a, b; van den Bergh and Snapper, 1913) on the other hand, he pointed out, had been shown to be sensitive to bilirubin /

bilirubin in a dilution of 1 in 1½ million in the blood serum. Previous studies had also suffered from the defect that only the urine was examined during the early stages after obstruction and he added that while the bilirubin threshold of the dog's kidney is very low this may in itself have been a source of error. Accordingly he removed both kidneys and the gall bladder from his dogs and ligated and divided the common bile duct. Having made a thoracic duct fistula he examined the blood and lymph at fifteen-minute intervals, using the van den Bergh test. The animals were maintained under anaesthesia. He came to the conclusion that, "during the first hours after occlusion of the common bile ducts, the bile pigments are carried from the liver by the lymph stream ....'. In a control series, having had only cholecystectomy and ligation and division of the common bile duct, he found that during the first two days the blood serum gave only the indirect reaction. From histological studies he reported that he could find no evidence of rupture of the bile canaliculi during the early stages of obstructive jaundice.

Another important paper appeared in 1928. In this Barron and Bumstead drew a similar conclusion from their experiments, namely that bile first appeared in the lymph but that the presence of a thoracic duct fistula only delayed bilirubinaemia /

bilirubinaemia for a few hours. They found, as did Bloom (1923), that the indirect van den Bergh reaction was observed during the first few hours in the blood of obstruction. Then the biphasic reaction occurred and some hours later the direct reaction 'which remains during the whole period of obstruction'. They believed that the indirect reaction which was present during the early phase could best be explained by assuming that biliary obstruction produced a nervous reflex which led to a state of paralysis of the function of the liver cell. As a result the circulating bilirubin remained in the blood stream. It was some hours before the cells resumed their function and again began the conversion of bilirubin into the direct-reacting type. They added, 'This is a field in which suggestions only can be made'. They concluded that, after biliary obstruction, bile enters the circulation both by way of the blood capillaries and the lymphatics, but that the latter route is the more important.

Aschoff (1932) was of the opinion that regurgitation into the lymph occurred in the region of the canals Hering or of the cholangioles.

Watson contributed an important paper in 1940 in which he pointed out that from the work of Bloom (1923) and of Hiyeda (1925, 1927) regurgitation of bile might occur in one /

one of two ways. By rhexis of the small biliary radicles, in particular, of what he describes as 'the weaker ampullary portions of the bile capillaries', allowing the bile to gain access to the adjacent lymph spaces or, alternatively, by 'increased permeability' of the bile capillaries with leakage or diapedesis of bile into the lymph spaces. He claimed that the ampullary portions of the bile capillaries appeared to be the areas most susceptible to injury and 'hence the chief sites of increased permeability and regurgitation'. The work of Hiyeda (1925, 1927), however, seems rather to have led to the conclusion that rhexis did not occur during the early stages of obstruction. Watson's paper contains no experimental evidence to support his views. Again in 1955, writing in Cecil's Textbook of Medicine he remarks that as far as can be determined obstruction of the common bile duct results in regurgitation jaundice because of injury to many of the smallest bile ducts within the liver. He added that 'Bloom (1923) and others have shown that the bile escapes primarily into the adjacent lymphatic spaces and thence to the thoracic duct and circulating blood.'

Weiss, in 1944, had this to say, 'in the obstructive form excess bile is found in the blood because there is increased /

increased permeability or rupture of the capillaries with leakage of bile into the lymph spaces. The bile is then reabsorbed, or regurgitated, through the lymph system.' It is possible that he means reabsorption into the blood but it seems more likely that he favours the lymphatic pathway.

In 1946 Gonzalez-Oddone, working in Watson's laboratory, brought forward the most convincing evidence hitherto produced in support of the lymphatic regurgitation theory. Unfortunately, it is not clear from the data presented how many dogs were used for each study. It seems almost certain however that since only a total of five dogs was employed and since the experiments described are of a varied nature only one dog was used for the bilirubin and one for the B.S.P. experiments. In these two animals the thoracic duct was canulated at its entrance to the jugular vein. Biliary obstruction was produced by dividing the common bile duct and tying the cystic duct. Data are given of a bilirubin study on one such preparation. Within thirty minutes the concentration of bilirubin in the thoracic duct lymph rose to 5 mg. per cent. During the next six hours it increased slightly and then began to fall gradually so that by seventeen hours it was lower than the plasma bilirubin concentration which had now reached 3.5 mg. per cent. From then until twenty-four /

four hours, which appears to have been the end of the experiment, the plasma concentration of bilirubin remained the higher. This was the first study in the unanaesthetised animal and, therefore, was of considerable interest, particularly because of its duration, and also since it showed that in this animal at least the throwback of bilirubin into the lymph was limited to the first twenty-four hours after obstruction.

A further experiment to study the effect of a single injection of B.S.P. was carried out and will be described below in the section of this review which describes the literature on B.S.P. studies during biliary obstruction. He concludes that his results support the belief that bile regurgitates into the lymph following common duct ligation and is carried thence via the thoracic duct to the blood.

With the development by Grindlay in 1946 (1948, 1950) a technique for inserting a polyvinyl tube into the liver lymphatic trunk and bringing it out through the parietes it became possible to establish a liver lymphatic fistula in the dog. In his Mayo Foundation thesis (1947) Cain described in detail some experiments which were carried out during biliary obstruction and which were only summarised in later publications. He made simultaneous studies of the bilirubin /

bilirubin content of lymph coming from both a liver lymphatic and a thoracic duct fistula in two dogs, under anaesthesia , after cholecystectomy and ligation of the common bile duct. The experiments lasted for four hours and one and a half hours respectively. In the first dog the bilirubin concentration rose from 1.2 mg. per cent. to 2.4 mg. per cent. within the first half hour. It remained at this level for the next three hours or so and then rose to 3 mg. per cent. No further readings were made. During this time the concentration of bilirubin in the thoracic duct lymph and in the blood plasma showed no appreciable change. In the second experiment the bilirubin level in the liver lymph rose from 3 mg. per cent. at half an hour after obstruction to 5.5 mg. per cent. at approximately one hour after obstruction. No further readings were reported. During this time the concentration of bilirubin in the lymph from the thoracic duct fistula rose from 0.8 mg. per cent. to 1.5 mg. per cent.

Watson's (1940) views were accepted by Ducci (1947) when, on the subject of regurgitation jaundice, he wrote, '..... its mechanism and features are well known and it is unnecessary to dwell on them'. He offered no experimental evidence to support this view. In the same year Mixer and his colleagues (Mixer et al., 1947) published an interesting paper /

paper describing the injection of diodrast into the common bile duct of dogs at what they termed, 'a very modest increase in pressure.' No data are given but they discovered the diodrast soon afterwards in the bladder. They asserted that there must have been rupture of the bile canaliculi to allow the passage of this substance into the blood, since its insolubility would have precluded simple absorption through the mucosa of the biliary tract. They also demonstrated thorotrast in the thoracic duct lymph and in the reticulo-endothelial cells of the spleen in dogs with thoracic duct fistulae, after retrograde bile duct injection. They regarded this as evidence that it had regurgitated into both lymph and blood. They then injected a solution of radio-active phosphorus in the same way and demonstrated its presence in both lymph and blood. The peak level of radio-activity in the lymph was found to be higher than that in the blood, but they stated that both levels eventually came into equilibrium. The experiments were done under anaesthesia and the longest time of study was five and a half hours.

In a critical review of the mechanisms of jaundice production Young (1947) asserted that during biliary obstruction the canaliculi are overfilled with bile, 'the tips of the canaliculi /

canaliculi bulge into the tissue space and bile diffuses into this space and thence to the lymphatic vessels and to the general circulation via the thoracic duct'. He quoted no experiments in support of this. A similar view was offered by Hanger (1950) who believed however that rupture of the intralobular and perilobular biliary channels took place and allowed all the constituents of the bile eventually to gain entrance to the blood stream.

Estimations of the levels of bile acids in thoracic duct lymph and blood in four cholecystectomised animals with biliary obstruction were made by Reiners in 1951. The measurements are tedious, taking about twenty-four hours for each sample, using the method he described (Irvin, 1944). Samples were taken, however, at hourly intervals and the animals were maintained under anaesthesia until the experiment had to be abandoned after eight to twelve hours because of dehydration. The data given shows an immediate rise in the concentration of bile acids in the lymph to levels of over 20 mg. per cent. within two hours of obstruction. The blood concentration rose steadily and by twelve hours exceeded that in the lymph, which had now fallen to about 12 mg. /

mg. per cent. They concluded that 'the preferential route through which the bile acid reaches the blood stream is through the lymphatics.' Similar observations of increases in bile acid concentration after ligation of the common bile duct had been observed by Junger et al. (1938) and by Chabrol et al., 1941, though the levels obtained by these workers were not reached as rapidly as in Reiner's experiments (1951).

An interesting contribution from Italy was made by Caldini and Montagnani in 1953. They quoted Dominici and Bruzzone (1938) as having shown that during biliary obstruction the thoracic duct contains high concentrations of bile. For their part they studied six cholecystectomised dogs with thoracic duct fistulae and followed them, under anaesthesia of an unspecified type, during the first twenty-four hours of obstruction. They concluded, 'La ligatura del coledoco e seguita da un brusco aumento della concentrazione di dette sostanze con un massimo in correspondenza delle 2^{A}-5^{A} ora.' Their data were based on bilirubin measurements of two hourly samples taken from the femoral vein and the thoracic duct fistula. From this they concluded that during the first twenty-four hours after obstruction the reabsorption of the constituents normally secreted in the bile is carried on mainly by the lymphatics.

In /

In 1953 Kirshen stated that during obstruction bilirubin escapes into the periportal spaces following damage to the biliary capillaries and enters the general circulation via the lymphatics. Lichtman (1953), however, in the same year limited himself to the remark that 'a lymphatic shunt of bilirubin and bile salts immediately follows even slight biliary obstruction.' Wintrobe (1954) accepted the view that rupture of the bile capillaries or damage to these structures leading to increased permeability formed what he described as 'the pathologic basis of regurgitation jaundice.' He added that the bile probably gains access first to the spaces of Dissé and that it is carried thence to the thoracic duct and the blood.

In 1956 Friedman and his colleagues described the effect of biliary obstruction in six rats, having liver lymphatic fistulae established by a method similar to that described by Bollman in 1948. They collected the lymph for twelve hours and stated that the cholate content was tremendously increased. They added that 'although no direct measurements were made, biliary obstruction was followed invariably within ten minutes by the appearance of a yellow pigment (apparently bilirubin) in a previously colourless lymph fluid.'

Regurgitation /

Regurgitation into the Blood

Of the remaining theories on the pathogenesis of the jaundice in biliary obstruction the most widely accepted holds to the view that bile is regurgitated from the biliary tree directly into the blood passing through the liver.

Two years after Harley published his paper in 1893, claiming that the lymphatic pathway was the more important, Lepine and Aubert (1885) stated that in their view regurgitation took place only into the blood. Since then the lymphatic theory has been criticised extensively. In 1894 Starling and Tubby injected indigo carmine and methyl blue into the pleural and peritoneal cavities of animals and noted that the colour appeared in the urine long before it was seen in the thoracic duct. They concluded that absorption of a substance into the blood was much more efficient and questioned whether any passed directly into the lymph, since the colour seen later in the lymph might well have diffused there from the blood itself.

Dastre (1897) brought forward the findings of Tobias (1897) as evidence against Harley's views. Tobias showed that the introduction of sodium ferrocyanide, strychnine and atropine into the bile ducts was followed by absorption into the blood.

The /

The first major experimental onslaught on the theory of regurgitation into the lymph was made in 1898 and 1899 by Wertheimer and Lepage. They performed experiments on dogs with thoracic duct fistulae. Ox bile was injected into the right hepatic duct and after a time they were able to observe the cholohaematin spectrum in bile coming from the left hepatic duct. They did not look for the pigment in the lymph because they found that by the time it appeared in the bile the lymph had already become red with haemoglobin. They then injected solutions of pure bilirubin under pressure into the bile ducts and obtained a positive Gmelin reaction in the lymph one hour later and in the urine after an interval of two and a half hours. They concluded: 'La verité est que les deux ordres des vaisseaux contribuent à leur resorbtion.' In the more recent paper they described how they had performed simultaneous ligations of the common bile duct and the thoracic duct and observed the appearance of bile in the urine. They had already shown that bile pigment appeared in the urine when the common duct alone was tied, and therefore they considered the lymphatic route to be relatively unimportant. Next they injected indigo dyes into the bile ducts observing that the urine was discoloured some minutes before the lymph. Accordingly they assumed that the pigment had passed into the blood and /

and been eliminated by the kidneys at a time when none of it could be seen in the lymph. They added, 'these few examples are enough to prove not only that the blood vessels take an active part in the absorption of the blue dye but also that the lymphatics play only a modest part in this process. The quantity of dye which passes through the thoracic duct is so small that it is often difficult and sometimes well nigh impossible to perceive a change in the colour of the lymph.' They offered no histological evidence as to the amount of damage caused by their injections into the bile duct.

In 1905 a similar series of experiments were carried out by Mendel and Underhill. They injected indigo carmine, potassium iodide, milk and iodine and milk containing potassium ferrocyanide into the common bile duct of dogs. They noticed that with the exception of the milk and iodine mixture these substances appeared in the urine before they could be detected in the lymph. They concluded that the hepatic blood vessels were the important factor in the absorption of such substances. McMaster (1923) however, did not observe bilirubinuria until twelve hours after obstruction.

Perhaps the most comprehensive study of this problem was carried out in 1911 by Whipple and King who extended their experiments to include the study of chronic biliary obstruction. The importance of their findings seems manifest but little attention /

attention appears to have been paid to their work in the past. With the animal under anaesthesia they ligated the common bile duct and also the thoracic duct at its junction with the jugular vein and were able to identify bile pigments in both urine and lymph by use of the Salkowski test. From these acute experiments they concluded that in obstructive jaundice, bile which escapes from the liver is absorbed by the blood capillaries and carried to the kidneys. As they put it, 'At best the lymphatic system is a secondary factor in the mechanism of jaundice.' Now they embarked on a most interesting experiment which is perhaps best reported in their own words: 'We have tried to simulate as closely as possible the conditions found in acute obstructive jaundice, have recorded our observations upon the lymph and urine and then permitted the animal to live for some time. After an interval of days or weeks the animal was examined for the presence of bile pigments in the various body fluids and this throws some light on the pathogenesis of chronic jaundice. It is believed that such experiments, extending over a long period of time, are more valuable than shorter ones in which no aseptic precautions were taken and in which the animal was sacrificed at the end of a few hours.' Briefly their findings were as follows:- The presence of a thoracic duct fistula /

fistula did not prevent the development of jaundice after biliary obstruction and that bile pigments sufficient to give a positive Salkowski test were only present in the thoracic duct lymph in some of their experiments. In the chronic experiments the lymph and pericardial fluid contained much less bile pigment than was found in the blood serum or urine. They concluded: 'It seems clear that in both acute and chronic obstructive jaundice the lymphatic apparatus takes no essential or active part in the absorption of bile pigments from the liver.

In 1927 Bollman, Sheard and Mann published some results of their studies. Bollman had introduced the Keuffer and Esser spectrophotometer for the measurement of bilirubin and this constituted a considerable advance, permitting relatively accurate measurements to be made for the first time. Their first method of study was to ligate the common bile duct in a group of dogs, remove the gall bladder and measure the bilirubin content of the blood at intervals thereafter. They found an increase in the concentration of bilirubin in the blood within five minutes, but added that the rate at which jaundice develops varies widely in different animals. They then found that if the gall bladder was present jaundice did not /

not develop until the concentrating activity of this organ was overcome. This they state usually occurred in from forty-eight to fifty-six hours. They believed that as soon as the pressure in the biliary ducts had risen to 300 mm. of water the hepatic cell became impervious to bilirubin so that bile pigment was neither excreted into the bile capillaries, nor removed from the blood by the hepatic cell. Their evidence for this was that if the liver had continued to excrete bilirubin or the hepatic cells to absorb it from the blood then some time would have elapsed before the blood concentration rose after obstruction. They pointed out that later, when the liver had become saturated with bile pigment, one would have expected a sharp rise in the bilirubin content of the blood. Whereas, in fact, an increase in the blood bilirubin concentration occurred a few minutes after obstruction had been produced and this increase was slowly progressive, there being no evidence of a rapid rise at any stage. They concluded: 'The present conception that the direct reaction is produced by bile pigment which is reabsorbed from the liver must obviously be incorrect if the hepatic cell does not withdraw this pigment from the blood.'

Regurgitation into Both Lymph and Blood

This /

This theory has come into prominence within the last thirty years, and represents perhaps the most balanced view which can be taken from the evidence available. It was first put forward by Mayo and Greene in 1929, who studied the effect of ligation of the common bile duct and the cystic duct on dogs under Amytal anaesthesia. The animals were divided into three groups, some having thoracic duct fistulae, some having the thoracic duct ligated and the remainder being used as controls. Samples were withdrawn at thirty-minute intervals and their content of bilirubin and bile acids was measured. The experiments lasted for five hours. They found that in the control group of dogs bilirubin and bile acids accumulated in the blood after simple ligation of the cystic and common bile ducts. In the fistulae dogs, they stated, that they found an even more rapid accumulation of these substances in the lymph and that in these animals the fistulae seemed to delay the changes in the blood, but did not prevent them. Ligation of the thoracic duct produced some delay in the entrance of bile constituents into the blood stream, but not so markedly as did the thoracic duct fistulae. They concluded that during biliary obstruction bile passes into both lymph and blood and added that the lymphatic route is, 'possibly more active during the first few /

few hours of obstruction.'

In was in 1930 that Rich published his celebrated paper on "The Pathogenesis of the Forms of Jaundice". In this he reviewed the available evidence and attempted a classification of jaundice on the basis of whether or not the pigment responsible for the icterus had been excreted into the biliary tree. Of the van den Bergh test he had this to say: 'I shall not enter into a description of this now well-known test other than to mention that when it is applied to bilirubin which has not yet passed through the liver cells there occurs a delayed, or so-called indirect reaction; whereas, in contrast, bilirubin taken from the bile ducts or from the gall bladder or that regurgitated into the blood from the bile canaliculi, gives a prompt or direct reaction.' This seems to have been the first use of the word regurgitation, in this sense, in the literature. He took the view that when the van den Bergh test on the plasma gave the direct reaction this indicated that wholebile containing bile acids and cholesterol, as well as bilirubin, had been regurgitated into the blood stream. He believed, therefore, that it was 'perfectly clear .... that one is dealing either with obstruction of the ducts or with necrosis of the liver cells. For these are the two conditions which permit bile to escape from /

from the canaliculi into the blood.' He went on to elaborate his subdivision of jaundice into two main types, calling that variety which results from mere retention of pigment 'Retention' jaundice and that which he believed to be due to passage of formed bile from the biliary tree into the blood, 'Regurgitation' jaundice. He did not himself offer any experimental evidence for these views.

Recently Cappell (1951), Popper and Schaffer (1952) and Sherlock (1956) are amongst the authorities who seem to have accepted this view. Inglefinger (1956) had this to say: 'A regurgitation jaundice, on the other hand, is marked by the predominant increase of bilirubin D in the blood ... At the onset, of such biliary obstruction, bilirubin D regurgitates from the intrahepatic biliary channels into the liver lymph spaces whence it is carried to the blood by way of the thoracic duct. Subsequently it is probable that bilirubin D also escapes into the sinusoids directly.'

Direct Passage

A few authors believe that direct anatomical connections exist between the bile capillaries and the lymph spaces or blood vessels within the liver.

As /

As early as 1776 von Haller was writing on direct communications which he believed to exist between the bile ducts and the hepatic veins. He injected various fluids into the bile ducts and recovered them from the hepatic veins. From this evidence he believed that during obstruction of the common bile duct the bile passed directly into the blood.

More recently Nauwerck, in 1897, on the basis of microscopic studies, believed that the hepatic cell was pierced by fine channels through which, during biliary obstruction, the bile entered the blood directly. He believed that they discharged into the sinusoids.

The distinguished French authority, Pavel, published some interesting observations in 1949. During histological studies he believed he had found evidence that the biliary canaliculi open into the space of Dissé. He is inclined therefore to the view that during biliary obstruction bile passes directly into the lymph through anatomical channels which are already present. He gave no photo-micrographs to demonstrate his findings. On the other hand they have received considerable support recently from studies carried out by Rouiller, in 1954, with the electron microscope. Rouiller's paper included some interesting photographs which he believed contained evidence that the biliary canaliculi open directly into /

into the space of Disse and also that the sinusoidal epithelium contained hiati whereby it also had free communication with the space of Disse.

Paracholie

A different trend of thought on this problem was introduced in 1892 (1895,1904) by Minkowski. His histological studies had led him to believe that damage to the liver cells occurred early during obstruction. He could find no evidence of canalicular rupture and became convinced that this damage produced a reversal of secretion whereby bile passed from the hepatic cells directly into the perisinusoidal spaces rather than into the bile capillaries. The theory has been discussed but not accepted frequently in the ensuing literature and has come to be known by the term 'Paracholie'. Minkowski argued that there was evidence available that the liver cell could function in two different ways. Not only did it excrete bile into the canaliculi but, as Claude Bernard (1855) had shown, the liver cell could secrete glycogen into the sinusoids. It was known that uric acid was handled in the same way and Minkowski asked 'why not bilirubin?'. He compared this alteration of normal function with that which occurs in the renal /

renal epithelium, on occasion, and which permits the escape of albumen into the urine.

In 1911 Sterling believed that he had observed early changes in the liver cells during obstruction and was also unable to find evidence of rupture of the bile capillaries. He therefore arrived at the same conclusion as Minkowski.

Other Theories

In the face of the plethora of theories described above it is perhaps not insignificant that the views of some distinguished workers in this field have yet to receive mention.

Some interesting experiments have been described by Shafiroff et al. in 1939. They were carried out on 28 dogs under nembutal anaesthesia. After ligation of the cystic duct bile from the animal's own gall bladder diluted with two volumes of normal saline solution was poured into its bile duct. The thoracic duct was canulated in the cervical region and quantitative van den Bergh estimations were made on lymph and blood at ten to twenty minute intervals. In the first group the intrabiliary pressure was maintained below the secretory pressure of the liver and here bile secretion continued. No bile was found in the lymph or blood. In the second group the pressure was maintained at about 300 mm. of water /

water and, in these, bilirubin appeared first in the lymph and in greater concentrations than were found in the blood, which showed no evidence of the pigment until fifteen minutes had passed. In the third group the intrabiliary pressure was kept above the secretory pressure of the liver and bilirubin was found in both lymph and blood within a few minutes. They stated, 'It is an interesting fact that while the bilirubinaemia resulting from obstructive absorption tended to attain a common level, irrespective of the degree of biliary pressure the concentration of bilirubin in the lymph appeared to vary with the degree of intrabiliary pressure. The mechanism underlying this phenomena is not yet clear. They drew no inference from these results.

In 1942 Shafiroff et al. found that with complete obstruction of the thoracic duct the concentration of bilirubin in the blood during biliary obstruction rose more rapidly and was much greater than was the case when the thoracic duct was only partially blocked. When no lymphatic block was present the blood level was even lower. Complete stoppage of lymph flow by ligation of the thoracic duct produced a marked reduction in the secretory pressure of bile in the extrahepatic ducts and when biliary obstruction was produced in these preparations, the bile ducts did not dilate in four out of the five dogs used.

With, /

With, in 1947, expressed dissatisfaction with the current views and stated on theoretical grounds that the jaundice which follows mechanical obstruction of the biliary tree must be of the retention variety because of the chemical constitution of the pigments which accumulated in the blood during the early stages. To explain the presence of bilirubin in the lymph at this time he postulated that 'active secretion' of bile might occur from the blood in the liver into the lymph spaces. He believed that true regurgitation from the biliary tree occurred in parenchymatous disease only.

Klatskin (1948, 1949) stated that studies in his laboratory had failed to confirm Watson's hypothesis that during biliary obstruction bilirubin is regurgitated from the biliary tree. He remarked: 'The concept that the prompt direct-reacting bilirubin has traversed the liver cells and has been regurgitated, is an assumption that still awaits proof'.

Bromsulphthalein /

Bromsulphthalein Studies

The literature concerned with the fate of injected B.S.P. (sodium phenoltetrabromophthalein disulphonate) during biliary obstruction is now reviewed. In 1913 Rowntree et al. and Whipple et al. (1913) introduced the halogenated phthaleins to estimate hepatic function. At first it was the practice to base calculations on the amount of B.S.P. found in the faeces (Kahn and Johnston, 1915; Krumbhaar, 1914; Rowntree et al., 1913; Sisson, 1914), this being taken as an index of how much was excreted in the bile. The method was not widely accepted but in 1924 and 1925 Rosenthal and White introduced the idea of determining the degree of retention in the plasma, after a standard dose per unit of body weight, to estimate hepatic efficiency. In this form the test has been employed extensively.

B.S.P. has been used experimentally by many workers in the study of liver function under various conditions and a vast literature has already been compiled in this way. Such of it as pertains to this investigation will be considered here.

In 1925 Snell et al. gave repeated doses of B.S.P. to dogs with biliary obstruction, but could find no dye in the bile /

bile passages of these animals. Again in 1927 Snell et al. observed that undue retention of B.S.P. in the plasma occurred during obstructive jaundice once clinical icterus had become apparent. Rosenthal and Bourne (1928) found that two to four hours of anaesthesia produced retention of B.S.P.

Mills and Dragstedt (1936) in some canine experiments confirmed Snell's findings. In the same year Dragstedt and Mills shows that intravenous injection of bilirubin interfered with the removal of B.S.P. from the blood.

The so-called 'lag phase' was studied by Wirts and Cantarow in 1942. The term refers to the interval occurring between the time of injection of the drug into the blood stream and that at which it first appears in the bile. They found it to be composed of two stages. Immediately following the injection B.S.P. was rapidly removed from the blood. They believed that this was effected by the Kupffer cells. Later, after the lag, came excretion into the bile. This they believed was done by the hepatic cell. Their views were later accepted and confirmed by Brauer and Pessotti in 1949, using rat liver slice and rat liver perfusion techniques.

In 1945 Bradley claimed that in the blood B.S.P exists in a state of chemical combination with the serum albumen. This has since been confirmed by Inglefinger et al., (1948) and

Cohen /

Cohen et al., (1954)

At the time of writing it seems that only one experimental study on the distribution of B.S.P. in the blood and thoracic duct lymph during biliary obstruction has appeared in the literature. Part of this paper, by Gonzalez-Oddone in 1946, has already been reviewed above. He described one experiment on the effect of a single injection of B.S.P. in a dose of 2 mg. per kilo given intravenously to a dog. The cystic and common bile ducts had been tied, and the thoracic duct canulated at its entrance to the jugular vein. The injection was given two hours after ligation of the common bile duct and the experiment was repeated thirty-four hours later. From the diagram given it can be seen that after the first injection of B.S.P. the concentration of the drug, in the thoracic duct lymph, rose to 4 mg. per cent. at the end of one hour and then fell to 1.3 mg. per cent. after two hours. The fall continued and, at six hours, the lymph contained 0.5 mg. per cent. No further readings are recorded. In the meantime the plasma concentration of B.S.P. at thirty minutes after the injection had risen to about 0.6 mg. per cent. It then fell slowly and by six hours it contained 0.4 mg. per cent. In the thirty-six hour injection no scale is given relating to the concentration of the drug, but the diagram /

diagram shows a high initial plasma concentration rapidly falling within two hours to reach that of the lymph which meantime had risen very slowly to this level. During the next three and a half hours a fall in the concentration of B.S.P. in the lymph is recorded. No further measurements appear to have been made. He stated that in the early period of obstruction, 'it is seen that the dye disappears promptly from the blood to be regurgitated at high concentrations into the thoracic duct lymph. In the later period of obstruction however the dye is retained in the blood and little or none appears in the thoracic duct lymph.'

In 1947 Cohn et al. showed that B.S.P. was removed from the blood in the hepatectomised animal and, in 1948, Cantarow et al. demonstrated that at elevated serum bilirubin concentrations bilirubin and B.S.P. compete for a common excretory mechanism into the bile. Brauer et al. (1950) produced evidence that B.S.P. may be altered chemically by the liver before it is excreted into the bile. They did not specify the changes involved. Retention of B.S.P., lasting for six days, in some cases of acute hepatitis and for twenty-eight days in one case of biliary obstruction due to carcinoma of the pancreas was reported by Giges in 1951. In the same year Zieve et al. accepted the hypothesis that B.S.P. regurgitates /

regurgitation from the biliary tree during biliary obstruction but presented no experimental evidence to support this view.

Norcross et al. (1951) demonstrated that the kidney is able to excrete significant amounts of B.S.P. when the serum concentration remains elevated for prolonged periods. Finally, Pratt (1952) in the course of liver blood flow experiments with B.S.P. on two anaesthetised dogs having liver lymphatic fistulae stated that they found the concentration of B.S.P. in the liver lymph to lie between that found in the portal vein and that in the hepatic vein. No measurements were given.

It is clear, therefore, that the manner in which B.S.P. is handled by the body, and by the liver in particular, has striking similarities to the treatment received by its more physiological counterpart bilirubin.

Fluorescence Microscopy During Biliary Obstruction

This technique also has yielded controversial results. Ansorge (1933) ligated the common bile duct of frogs at its entrance to the duodenum and injected fluorescein intravenously. She observed dilatation of the bile canaliculi within /

within the first twenty minutes. The canaliculi were well filled but within the next ten minutes she noticed that the fluorescein disappeared from the canaliculi and seemed to enter the hepatic cells. This was the first time the cells were seen to contain fluorescein. It now appeared to leave the hepatic cell and pass into the blood. In the same year Markstahler carried out similar experiments in the rat. Fifteen minutes after the injection fluorescein appeared in the canaliculi. During the next thirty minutes or so it could be seen in vacuoles in relation to the canaliculi. These vacuoles then appeared to burst and the dye flowed into the hepatic cells. At this stage she noticed that none was visible in the canaliculi. The dye was then seen to pass from the hepatic cells into the blood. In her experiments also the dye appeared in the biliary canaliculi before it was seen in the hepatic cell.

In 1938 Hirt, Ansorge and Markstahler published a paper which merely repeats the findings described above. Hanzon (1952) made observations on rats, under barbiturate anaesthesia, during the first thirty-six hours after obstruction of the common bile duct. He injected fluorescein intravenously and found generalised dilatation of the bile capillaries, /

capillaries, ruptures, increased permeability of the hepatic cells and leakage from the canaliculi into the blood. The most authoritative account of this subject available in the literature was published by Grafflin and Chaney in 1953. They injected fluorescein into mice and found no evidence of dilatation of the bile canaliculi, canalicular rupture or leakage of the dye from the biliary tree into the blood. They showed that many of the phenomena described by previous authors such as 'sprouting', 'intracellular vacuole formation' and 'imbibition cells' were artefacts. They claimed that these occur when the liver has been injured by excessive ultraviolet irradiation during the technique. With regard to the work of Hanzon they state that examination of his photomicrographs of the liver, during biliary obstruction, which he believed to show generalised dilatation of the canaliculi, had in fact revealed canaliculi which were of normal calibre. They pointed out that from Hanzon's own data in the earlier part of his monograph all of the effects he observed could be attributed to injury of the liver by excessive ultraviolet irradiation.

Recent /

Recent Advances in the Chemistry of Direct-Reacting Bilirubin

Within the last three years considerable progress has been made in our knowledge of the chemistry of direct-reacting bilirubin. It seems that the division of bilirubin into indirect and direct types on the basis of the diazo-reaction, discovered by Ehrlich in 1883, has more chemical than physiological significance.

Since 1900 when this azo-bilirubin compound was first isolated and studied chemically and spectroscopically by Prôscher, much work has been done on the chemistry of the pigment, but it was not until the work of Cole, Lathe and Billing, in 1954, that any clear idea of its significance could be obtained. They showed that it was possible, by chromatographic means, to fractionate direct-reacting bilirubin taken from the serum of patients with obstructive jaundice into two distinct moieties. This was possible since they travelled at different speeds through the column. They designated the slower moving band, pigment I, and the faster, pigment II. They found the proportion of each in such sera to be variable.

In 1956 Billing and Lathe showed that most of the bilirubin present in fresh human bile was in the form of the faster /

faster moving pigment II. This followed an experimental observation by Billing in 1955 that pigment II predominated in the serum of rats with biliary obstruction. Both observations have been confirmed by Hoffman et al. (1957) who also showed that pigment II predominated in fresh canine bile and comprised about 75 per cent. of the total bilirubin derivatives present. He found that pigment II predominated in the serum of patients with obstructive jaundice, particularly during the first week or two of this condition. Cases of acute hepatitis, however, and animals exhibiting jaundice due to toxic substances such as toluenediamine and ethionine showed a predominance of pigment I in the serum. So it appeared that the presence of liver cell damage produced an accumulation of pigment I in the serum. Whereas in biliary obstruction pigment II was more plentiful.

In their paper in 1956 Billing and Lathe showed that the bilirubin found in bile was a glycuronic acid conjugate. Since incubation for twenty-four hours with the enzyme and glycuronidase or mild alkaline hydrolysis freed glycuronic acid on a molar basis. This has been confirmed by Schmidt (1956). A subsequent demonstration by Bollman and Hoffman (1957) that pigment I accumulated in the serum of hepatectomised dogs has shown that it may be formed outside the liver /

liver and they believe that it is the function of the hepatic cell to change pigment I by further conjugation with glycuronic acid and to excrete it as the di-glycuronide of bilirubin in the bile. They have evidence that pigment I is, in fact, the monoglycuronide of bilirubin, as has Schmidt (Bollman, Personal Communication). It appears that Lathe and Billing take a similar view (Bollman, Personal Communication).

Significance of Previous Work

Biliary obstruction in man may occur under a variety of circumstances. Commonly it is seen when a non-functioning gall bladder is present or at some time after such a gall bladder has been removed. It frequently occurs when a functioning gall bladder is present. It is unusual to find it within twenty-four hours of cholecystectomy. A scrutiny of the literature however has shown that experimental work carried out on this problem has been confined to animals from which the gall bladder has been removed a few hours previously. Since the time of Oddi (Rous and McMaster, 1921) it has been recognised that profound changes in the biliary tree follow removal of the gall bladder, but that these take some weeks or months to develop. Consequently it seemed possible that the phenomena, /

phenomena which had been observed might not give a true indication of what happened during the majority of clinical cases of biliary obstruction.

If obstruction of the biliary tree in man should resolve it frequently recurs. No experimental studies have been described in the literature of the effect of a second period of obstruction.

Biliary obstruction may be acute or chronic. The available studies have, with the exception of the work of Whipple and King (1911) been confined to acute obstruction. Whipple and King, because of limitations in the biochemical techniques available in 1911, were not able to make any quantitative measurements.

In the past thoracic duct lymph only has been studied, with the exception of two experiments, lasting one and a half and four hours respectively, carried out by Cain, in which the liver lymph itself was studied. The flow from thoracic duct fistulae in dogs is such that within twenty-four hours 95 per cent. of the available plasma volume may be lost, (Nix et al., 1951). Hence it is not possible to prolong these studies beyond this time. Furthermore the liver lymph itself may be expected to give a more accurate representation of what is happening within the viscus.

Experimental studies on preparations with lymphatic fistulae described in the literature have, with one exception, been carried out on anaesthetised animals and so were of limited duration. In Gonzalez-Oddone's experiments, where a conscious animal was used, the study lasted for twenty-four hours only. A scrutiny of his data will show that by the time twenty-four hours had elapsed there was no evidence whatsoever that the concentration of bilirubin in the lymph coming from the thoracic duct fistula was higher than that in the venous blood. It seemed desirable therefore that the progress of acute biliary obstruction should be studied for a longer time than had been done in the past, and to do this it was essential to use unanaesthetised preparations.

Only one study has been reported of the effect of acute obstruction of the common bile duct on the levels of B.S.P. in the blood and thoracic duct lymph (Gonzalez-Oddone, 1946). It seems that the liver lymph has not been studied under these conditions. Studies of the distribution of B.S.P. in the lymph during chronic obstruction have yet to be reported.

No information is as yet available on the type of direct pigment which appears in the lymph coming from the liver after acute obstruction and it seemed possible that additional information on this subject might be obtained by subjecting samples /

samples to 'Reverse Phase' chromatography.

Detailed Statement of the Problem to be Investigated

It was the aim of this investigation to study in the unanaesthetised dog the development of the jaundice which follows complete mechanical obstruction of the common bile duct. It set out to measure the changes produced thereby in the concentration of bilirubin in the venous blood and in the lymph coming from the liver. An attempt was made to produce experimentally circumstances similar to those which obtain during biliary obstruction in man.

It was proposed therefore to study in this way:-

(1) The effect of acute obstruction of the common bile duct,
   (a) in the recently cholecystectomised animal:
   (b) in the previously cholecystectomised animal:
   (c) in the animal with its gall bladder *in situ*.

(2) The effect of a second period of acute obstruction in the cholecystectomised animal.

(3) The effect of chronic obstruction of the common bile duct in the cholecystectomised animal and in the animal with its gall bladder *in situ*.

It was then proposed to repeat experiments Nos. 1 (a), 2 and 3 on dogs which were being given a continuous intravenous injection of B.S.P.

To investigate the possibility of a species difference, similar bilirubin studies of the effects of acute and chronic biliary obstruction were to be carried out on rats with liver lymphatic fistulae.

In this way it seemed that it might be possible to establish the Standard Response to Biliary Obstruction in terms of the changes it produced in the concentration of bilirubin, or injected B.S.P., in the circulating blood and in the lymph coming from the liver. If these patterns could be established it was proposed to carry out more intimate studies on the Mechanism of the Standard Response. In particular it was the aim to ascertain whether or not, during biliary obstruction, bilirubin and B.S.P. were excreted into the biliary tree. Finally, an attempt was to be made to identify the direct pigments present during obstruction in the liver lymph by the method of 'Reverse Phase' chromatography, so that further information could be obtained as to whether or not they were likely to have come from the biliary tree.

MATERIALS AND METHODS

Description of General Materials Used.- Fifty-two healthy mongrel dogs, weighing 8 kg. to 12 kg., and 12 male albino rats of the Sprague Dawley strain, weighing 212g. to 270 g., were used for study in this investigation.

In the dog experiments polyvinyl catheters (Transflex size 20 and 24, Irvington Plastics, Irvington 11, New Jersey) were used. These are pliable and do not kink easily. They have the further advantage that the clotting of lymph in them is considerably retarded (Cain , 1947).

The Canine Experiments

Pre-operative Preparation

No special training was given to the animals. They were fasted for twenty-four hours before operation and hair was clipped from the abdomen and right hemi-thorax. The skin was prepared with surgical spirit and Merthiolate, 1 in 1,000.

Anaesthesia

This was induced in a specially reinforced glass anaesthesia box with ether and a suitable endotracheal tube was inserted. If a thoracotomy was being performed an automatic 'pulmoflator' /

'pulmoflator' of the type in use in the Surgical Research Laboratory of the Mayo Clinic was used. Ether anaesthesia was maintained throughout. All animals recovered consciousness within an hour after the end of the operation and usually within fifteen minutes.

Operative Procedures

All operative procedures were carried out in a fully equipped 'theatre' with aseptic precautions.

(1) A. Liver Lymphatic Canulation.- The technique used was a modification of that described by Grindlay et al., (1948, 1950). Through an incision in the right tenth interspace, from the middle of the right rectus muscle to the lateral edge of the paraspinal muscles, the chest and abdomen were opened. The lateral half of the right rectus muscle was incised to give full exposure. The diaphragm was split radially to the right leaf of its tendon and the incision carried laterally to the apex of this leaf. Peritoneal reflections connecting the under surface of the right lobe of the liver to the right kidney and the anterior surface of the inferior vena cava were divided and the liver gently rotated upwards and outwards. A moist pack was inserted above the diaphragm in the right side of the chest to damp down the excursion /

excursion of the right lung which tends to make insertion of the canula difficult.

A moist towel was now arranged to protect the liver which was rolled over the hiatus in the diaphragm on to it. The duodenum and its mesentery were swathed in a second towel. Traction was now exerted on this by an assistant to expose the liver lymphatic trunk for canulation (Fig. 1). The anatomy of these lymphatics as they were found at operation in 100 consecutive examinations of canine livers will be described in detail in Appendix A.

The main liver lymphatic trunk draining the hilar lymph was ligated where it joins the cysterna chyli on the medial side of the inferior vena cava (Appendix A.). This produced engorgement of the liver lymphatics and a suitable site was chosen for canulation. A No. 24 catheter was inserted and tied in position (Fig. 2). It has been found to be most satisfactory if the catheter is brought out through the parietes in the right loin just cephalad to the iliac crest and ventral to the lateral edge of the sacrospinalis muscle. A small plug of Ivalon sponge was tied to the intra-abdominal part of the catheter where it pierced the abdominal wall. This prevents any traction bearing directly on the lymphatic. As /

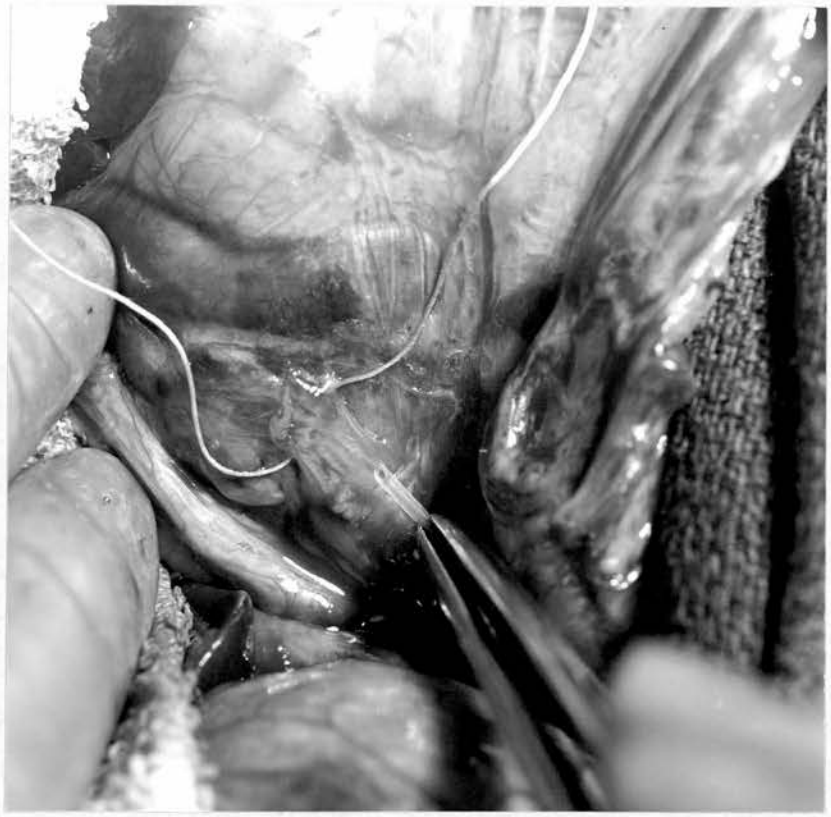


Fig. 1.- Canulation of the Main Hilar Lymphatic Trunk I
A ligature has been passed behind the trunk and a polyvinyl tube is shown held ready for insertion. Above (ventral) is the superior mesenteric vein and to the left (cephalad) is the hepatic artery.

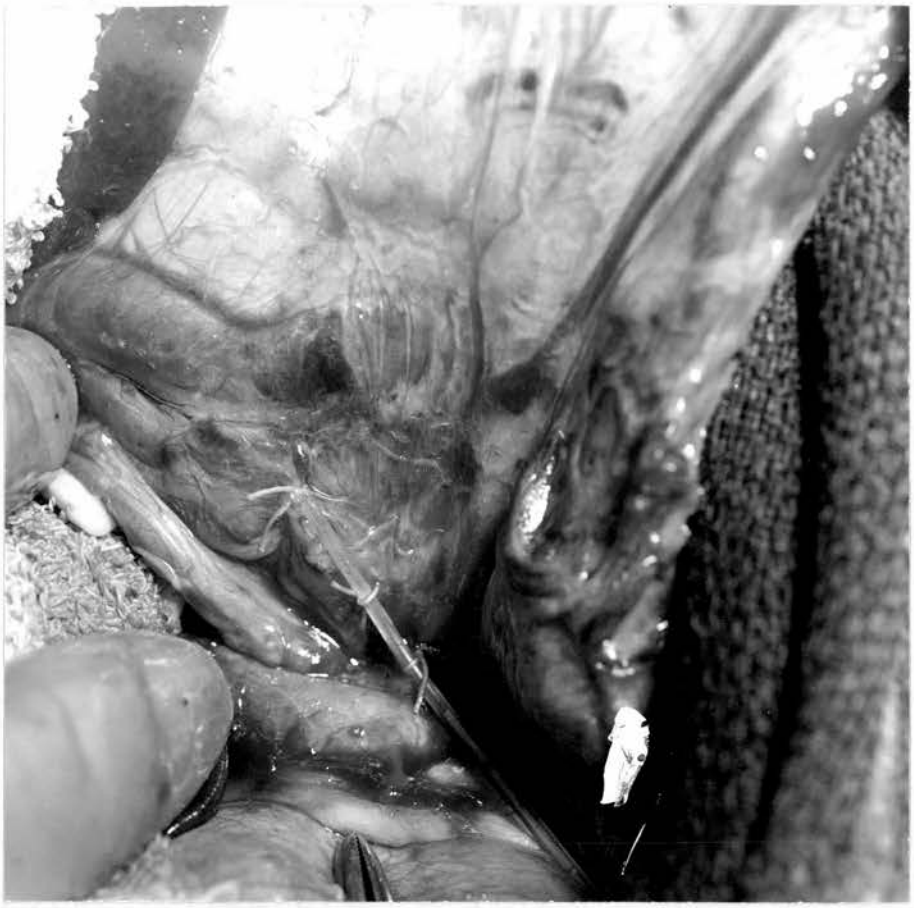


Fig. 2. Canulation of the Main Hilar Lymphatic Trunk II. The polyvinyl tube has been inserted and is shown tied into the lymphatic trunk.

As an added safeguard a rubber tube, one inch long, having quarter-inch bore, was stitched to the animals back, just dorsal to the point of penetration of the lymphatic catheter, which was now passed through the tube. This prevents the animal from rubbing the catheter against the wall of the cage, (Fig. 4.)

(1.) B. Thoracic Duct Canulation.- The thoracic duct was canulated in the chest two or three inches above the diaphragm. At this level in the dog it lies imbedded in the areolar tissue which is adherent to the under surface of the thoracic aorta. The free end of the catheter was then passed through a stab wound in the right dome of the diaphragm and out through another in the right loin, near the point of exit of the liver lymphatic canula.

(2) Cholecystectomy. Double Ligation and Division of the Common Bile Duct.- These procedures were carried out as the need arose in the classical fashion. Non-absorbable cotton ligatures were used throughout. Scrupulous care was taken to prevent any escape of bile into the peritoneal cavity, since it is rapidly absorbed from there by the lymphatics.

(3) Methods of Producing 'Delayed Obstruction'.- It is advantageous, in a study of this nature, to have accurate measurements of the concentrationsof the constituents under study, /

study, in bile, lymph and blood before, as well as during and after, obstruction. To permit of pre-obstructive control studies of this type both of the following methods of producing 'delayed obstruction' were employed, as the circumstances required.

Method 1.- The common bile duct was canulated with a No. 20 catheter which was brought out in the right loin through the abdominal wall. It could be occluded with a metal nail and released at will.

Method 2.- A heavy braided No. 5 silk 'snare', encircling the distal part of the common bile duct, had both ends passed through a stiff polythene tube, which was exteriorised through a stab wound in the right loin. The snare could be used later, after the manner of the Rumel tourniquet, to produce obstruction of the duct. This method was not used if obstruction was to be relieved after a period. Its effectiveness was checked after each experiment at post-mortem.

(4) Canulation of the Portal Vein.- In a few animals a No. 20 Transflex catheter was inserted into a splenic vein and advanced until its tip could be palpated as it lay in the lumen of the portal vein.

(5) Insertion of Venous Catheters for Sampling and Intravenous Injections.- No 20 Transflex catheters were inserted into /

into the leg veins and pushed up into the iliac veins or the caudal end of the inferior vena cava, for the purpose of taking samples or for intravenous injections, or both.

Post-operative Care

(A) The Handling of Animals with Catheters in situ.- It is essential that the tip of the lymphatic and biliary catheters be kept below the level of the animal's abdomen (Fig. 4) so that unobstructed flow may be maintained and clotting in the lymph catheter avoided. To assist in this the animals were placed in raised cages, of the type shown in Figure 3, which limited their activity. A six-inch broad malleable metal collar was put on the animal's neck to stop it turning its head in the cage and interfering with the catheters (Fig. 4).

A further advantage of the rubber tube stitched to the animal's back to act as a saddle was that it now allowed the lymph catheter to be passed through a hole high up in the wire mesh side of the cage and then downwards outside the cage to below the level of its floor (Fig. 4). This permitted the animal to stand, sit or lie down without kinking the catheter, which merely slid up and down above it with each movement. If a bile duct catheter was present it was also passed through the saddle and out through the side of the cage in the same way (Fig. 4).

(B) /

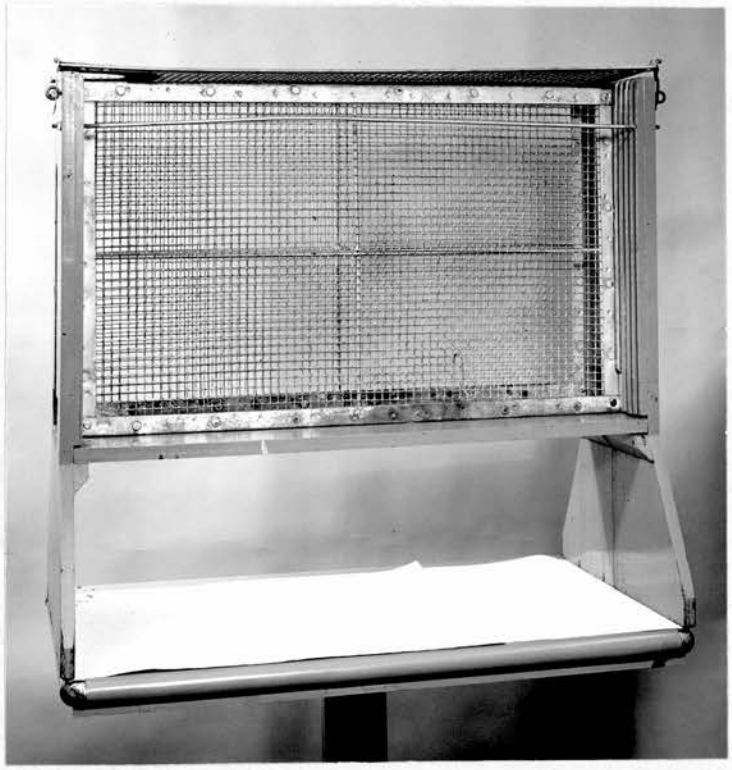


Fig. 3. A Cage which Limits the Activity of Dogs (Bollman, Personal communication)

The roof is hinged to allow the animal to be put into the cage from above. The front of the cage is in the form of a movable partition which can be adjusted according to the size of the animal.

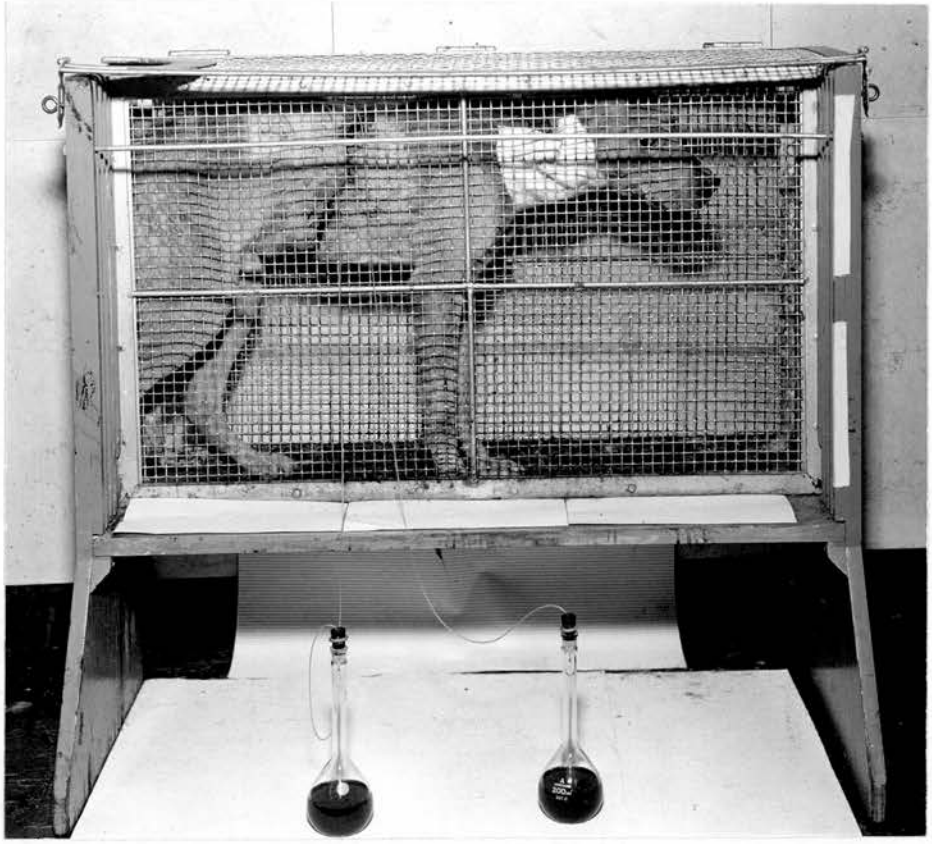


Fig. 4. An Animal in the Cage During a Typical Experiment

In this preparation polyvinyl tubes were inserted into both the liver lymphatic trunk and the thoracic duct. The tubes are passed through the wire mesh near the top of the cage. A thin metal collar, in a soft cotton 'pillow case', has been applied to limit turning of the head and neck.

(B) Feeding and the Maintenance of Fluid Balance.- After twenty-four hours from the time of operation the animals drank water freely and began to take milk and biscuit feeds. The flow of lymph and bile, if prolonged, constitutes an appreciable loss of fluid, protein and electrolytes. The discharge from such fistulae is discussed in Appendix B. In a 10 kg. dog it averages about 150 ml. per day in the presence of biliary obstruction (Appendix B).

It is not advisable to return this lymph to the circulation as vasoconstrictors and bacterial toxins are formed while it lies in the collecting vessel. These may cause vascular upsets in the liver and elsewhere if they are reinfused (Bollman, Personal Communication; Andrews et al., 1953a). The fluid loss in experiments lasting over twenty-four hours was, therefore, made good with continuous intravenous glucose and saline infusions of about 200 to 250 ml. per day. This was usually done by means of a constant speed injection apparatus of the type made in the engineering laboratory in the Mayo Clinic (Pages 71, 72). The animals passed urine freely throughout and remained in good condition. In experiments lasting for four days however they became listless on the fourth day. It was difficult to assess how much of this was due to protein deficiency, how much to the jaundice and the effects of prolonged biliary obstruction and how much to/

to confinement in the cage for this length of time. Every precaution was, however taken to keep the haematocrit steady and experiments were discarded if any significant change occurred.

The Rat Experiments

Anaesthesia

This was induced and maintained with ether.

Operative Technique and Maintenance of Fluid Balance

Canulation of the liver lymphatic trunk in the rat was carried out by Emery Van Hook, chief technical assistant to Dr. J.L. Bollman, to whom I am indebted for these preparations. The method used was that described by Bollman et al., (1949). Small P.E. 50 polythene tubes were inserted into the lymphatic and also into the tail vein. Saline was given throughout the experiment by a constant speed injection apparatus, in amounts of 2.5 ml. per hour. When obstruction was required the common bile duct was doubly ligated and divided at operation. The animals were kept in a specially designed cage which limits their activity (Fig. 5) (Bollman, 1948).

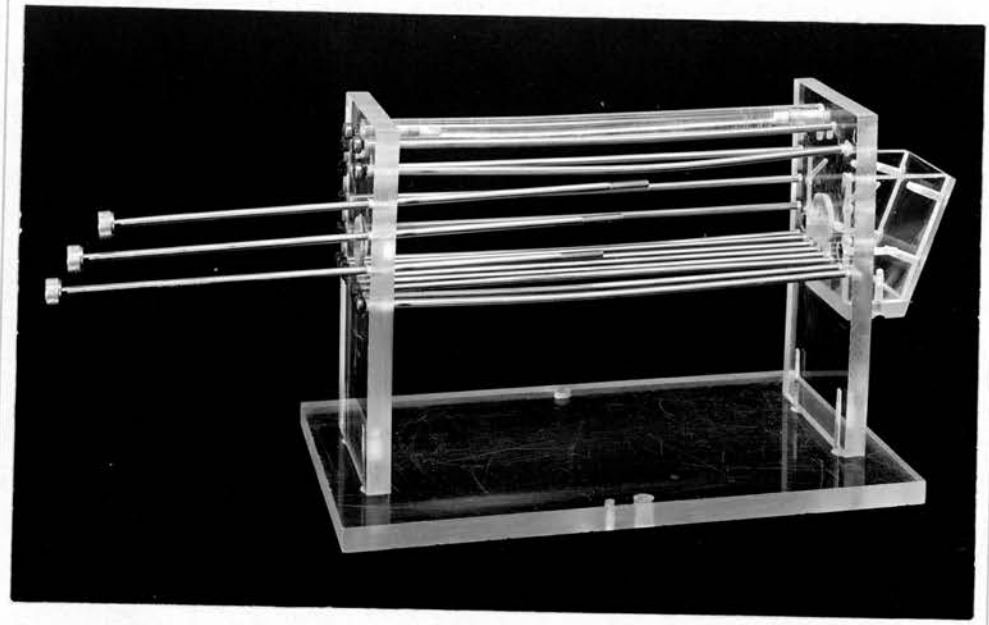


Fig. 5. A Cage which Limits the Activity of Rats (Bollman, 1948)

Three loose metal spars are shown. These can be removed to permit insertion of the animal into the cage and are then screwed home into the Perspex frame. The trough at the front (right) is for food.

Sampling Technique

The Canine Experiments

Lymph.- The samples were collected in heparinised 15 ml. calibrated 'pyrex' centrifuge tubes. A special perforated stopper was used so that the catheter could pass through with its tip hanging free in the lumen of the tube. This allowed the lymph to form droplets and avoided clotting against the wall of the tube. In such experiments it is seldom necessary to take for assay samples of more than 0.5 to 1.0 ml. volume. This amount of lymph can usually be obtained from the catheter in five to ten minutes (Appendix B).

Blood.- These samples were withdrawn at the mid point of the time taken for collection of the lymph sample. One to 2 ml. of blood was withdrawn into a syringe which was then detached and the blood discarded, a second clean heparinised syringe was now used to withdraw 2 to 4 ml. of blood for the sample. The catheter was then filled with isotonic saline, 2 ml. to 4 ml. of which was injected, and its tip occluded with a metal nail. The blood sample was then transferred to a pyrex centrifuge tube.

Bile.- Bile samples were collected from the common bile duct tube directly into a 15 ml. calibrated pyrex centrifuge tube.

The /

The Rat Experiments

Lymph.- Flow is much slower in the rat. In the animals used however a minimum volume of 0.3 ml. was being collected every thirty minutes. It was allowed to drain into heparinised pyrex tubes similar to those used in the canine experiments, and so placed that the tip of the catheter lay about one inch below the level of the animal's abdomen.

Blood.- Blood samples were withdrawn by direct cardiac puncture, under ether anaesthesia, at the end of the experiment.

All lymph and blood samples withdrawn during the canine and rat experiments were immediately centrifuged for ten minutes at 2,500 r.p.m. Note was now taken of the percentage of red cells to plasma in these graduated centrifuged tubes.

Chemical Methods and Materials

Bromsulphthalein (B.S.P. is sodium phenoltetrabromophthalein disulphonate) supplied by Hynson, Westcott and Dunning Inc., Baltimore, Maryland, was used throughout. It was given intravenously by a constant speed injection apparatus. Special syringes having a plunger travel of two and five-eighth inches were used and the apparatus was designed /

designed so that they would be emptied in one, four, eight, sixteen or twenty-four hours, according to the setting. The apparatus is calibrated regularly and is accurate to within 5 per cent. of the total volume to be delivered over these periods.

The Estimation of Bilirubin

This was carried out on lymph, blood and bile samples by the modification of the method of Malloy and Evelyn (1937) recommended by the American Association of Clinical Chemists (1953). Final samples were read in cuvettes 19 x 105 mm. in a Coleman 14 spectrophotometer at mμ 540. The concentration was determined from a previously constructed graph based on observations obtained with known concentrations of bilirubin. The results (see Protocols) are expressed in two figures for each sample given one above the other. The upper figure refers to the fifteen minute direct reading, and the lower the total bilirubin reading.

The Estimation of Bromsulphthalein

This was estimated in lymph and blood by the method of Gaebler (1945), and in bile by the method of Cantarow and Wirts (1941). Final samples were read in a Coleman 14 spectrophotometer at 575 mμ. in cuvettes similar to those used /

used in the bilirubin estimations. Here also the concentration was determined from a previously constructed graph based on observations obtained with known concentrations of B.S.P.

'Reverse Phase' Chromatography

Samples were examined by the method of Billing (1955a) This was carried out by Dr. H.N. Hoffman II to whom I am indebted for these observations. After chromatographic separation the pigments were diazotised and quantitated as in the bilirubin estimations (Billing, 1955a).

SOURCES OF ERROR

Errors Arising from the Animals Used

All animals were examined at operation. In particular, the liver and biliary tract were inspected. In some anomalies of the bile ducts were observed and precautions were taken to ensure that accessory ducts were not 'missed'. Occasionally the right lobe of the canine liver has a discrete duct which enters the duodenum independently of the common bile duct.

If the liver appeared abnormal the animal was not used. In /

In one of the canine experiments hepatitis was observed at operation and early cirrhosis was present in three. These were not included. In both the canine and rat preparations with chronic biliary obstruction biliary leaks with peritonitis were observed in several instances, at the second operation when the lymphatic canula was to have been inserted. Such experiments were abandoned.

It is possible that some sub-clinical hepatic or other lesion existed and could have influenced the results. The animals were, however, supervised in the kennels by a staff of veterinary surgeons and all reasonable precautions were taken.

A further examination was carried out at post-mortem. If the liver or other organs seemed to be the site of pathological change sections were taken for histological study. If any positive findings which might have influenced the results were made, the experiment was discarded. The opportunity was also taken to investigate the competency of all catheters. In particular, a leak during obstruction from the site of fixation of the common bile duct tube could have invalidated the results. Inspection of the peritoneal cavity for such an extravasation and testing of the common bile duct catheter were uniformly carried out.

Errors /

Errors Arising from the Techniques Used

(1) Those Arising out of the Operation Itself.- Excessive instrumentation and trauma to the liver may cause bleeding into the lymph. This dilutes the substance being measured and interferes with flow. It may promote clotting. Care was taken to minimise this. Significant bleeding into the lymph occurred in two canine and several rat experiments and these had to be abandoned.

(2) Those Arising out of the Lymphatic Canulated.- In a few canine preparations the lymph became milky and opalescent. This is due to chyle from the intestine and denotes contamination of the liver lymph with that coming from the bowel (Appendix A). Such preparations were not used.

There was no way of being certain of what fraction of the total liver lymph output was being collected. Some of the canine liver lymphatics follow the hepatic veins and enter the thoracic duct near the diaphragm (Grindlay, Personal communication; Bollman, 1950). It was clear that a loss was occurring in this way (Bollman, 1950; Morris, 1956). A control series was therefore investigated with simultaneous catheterisation of the thoracic duct and liver lymphatic trunk to evaluate this and the results reported in Appendix B.

(3) /

(3) Those Arising from the Lymphatic Catheter Drainage.- It was found that in a few canine preparations the flow from this catheter was small, being of the order of 2 ml. to 3 ml. per hour. Because of the possibility that unusually large amounts of the lymph might be travelling via the thoracic duct, the preparations were discarded.

If the clotting occurred in the canula attempts were made at once to relieve this by gentle suction. Unless the flow could be re-established within a few minutes the experiment had to be discontinued.

(4) Those Arising from the Sampling: A. Errors in the methods of collection.- All glassware used was carefully washed and dried by trained staff who are fully occupied in this way.

Contamination may also arise if old blood, which has been stagnating in the catheter since the time of taking of the previous sample, is included in the next. Accordingly, the first 1 ml. to 2 ml. of blood withdrawn was discarded and the catheter was refilled with saline after withdrawal of each sample.

There are errors which may arise during the withdrawal of samples of venous blood from small leg veins. Under these circumstances the blood is slow to withdraw and has come from

a /

a limb in which congestion and stasis have often been present because of bandages or surgical taping around the site of exit of the catheter. It was, therefore, the practice to push these catheters well up into the iliac veins or the caudal part of the inferior vena cava. This gave a free flow when 10 ml. syringes were used and blood could be withdrawn rapidly which is essential in B.S.P. balance studies.

To avoid contamination of a sample of blood during withdrawal with the B.S.P. which was being continuously infused into another part of the venous stream the infusion was stopped for about twenty seconds before withdrawal of the sample. It was also the practice to ensure that the catheter to be used for the infusion lay much nearer to the right atrium than that for withdrawing the samples, if these catheters were to be inserted into both hind limbs.

(B) Errors Arising from the Timing of the Samples.-
The time of withdrawal of the sample is critical in 'balance' experiments involving a continuous infusion such as the B.S.P. group. Since it is a straight comparison between the concentration of the substance under study, in the lymph leaving the liver, with that in the blood passing through the liver, which is being sought, it seemed most satisfactory to withdraw the blood sample as near as possible to the mid point /

point of the time taken for collecting the lymph sample. This method gives the average concentration over a five or ten minute period in the lymph and since the blood concentration should be increasing at a uniform rate a sample taken in the middle of this five or ten minute period should also represent the mean blood concentration for that time.

Greatest accuracy may be achieved by frequent sampling, provided the animal's blood volume is not thereby being significantly depleted. If this occurs the response will be altered. A close watch therefore must be kept on the haematocrit throughout such experiments. This can be done quite simply by observing the ratio of plasma to red cells after the routine centrifuging of each sample has been carried out. It was the practice in this investigation, once the general pattern in a series of experiments had been discerned, to limit the taking of blood samples to intervals during which, experience had shown, that no significant change could occur and be overlooked. Furthermore, since a large number of samples were being collected and assayed, the risk of mistakes and confusion arose. All tubes were clearly marked immediately after withdrawal to reduce this risk as far as possible.

While a series of dynamic studies, such as comprise this thesis, do not easily lend themselves to statistical evaluation, /

evaluation, they have this excellence, that each measurement is of itself controlled by those which precede and follow it (Berkson, Personal communication).

Errors Arising in the Chemical Methods

(1) Fresh reagents were used for each experiment. In particular, solutions of sodium nitrite for use in the bilirubin estimation, are prone to lose their potency.

(2) 'Blanks' of the actual sample being measured were used in the spectrophotometer on each occasion.

(3) Haemolysis occurred in the serum of a few canine preparations. This may affect the results of the bilirubin assay (American Association of Clinical Chemists, 1953). It usually cleared up within three to four hours and care was taken not to begin the study until this time. B.S.P. estimations, made by the technique of Gaebler (1945) are not significantly affected by this. Some isolated samples exhibited this change which must have been due to contamination of the glassware. These are denoted in the protocols.

(4) When the concentration of bilirubin or bromsulphthalein in the sample for assay is under 0.3 mg. per cent. the measurements become inaccurate. These are well recognised limitations of such techniques and little reliance can be placed /

placed on figures below this level (Flock, Personal communication). In the studies presented herewith the changes are usually so gross as to make these limitations in the measuring techniques used insignificant.

THE STANDARD RESPONSE

TO BILIARY OBSTRUCTION

THE CANINE EXPERIMENTS

When mechanical obstruction of the common bile duct is complete it may be acute or chronic. If it should resolve it may recur. To study the course of the jaundice in these types of obstruction the following plan of investigation was adopted:-

Bilirubin Studies

Acute Obstruction (pp. 84 - 106)	Recently cholecystectomised animal. Previously cholecystectomised animal. Animal with gall bladder in situ.
Recurrent Acute Obstruction (pp. 107 - 111)	Cholecystectomised animal.
Chronic Obstruction (pp. 112 - 117)	Cholecystectomised animal. Animal with gall bladder in situ.

B.S.P. Studies

Acute Obstruction (pp. 119 - 130)	Cholecystectomised animal.
Recurrent Acute Obstruction (pp. 119 - 130)	Cholecystectomised animal.
Chronic Obstruction (pp. 119 - 130)	Cholecystectomised animal.

Complete details of each experiment are to be found in the Protocols at the end of this thesis.

BILIRUBIN STUDIES

ACUTE OBSTRUCTION OF THE COMMON BILE DUCT IN THE RECENTLY CHOLECYSTECTOMISED ANIMAL

Acute obstruction of the common bile duct was produced in various ways in six animals (Nos. 1 to 6) and the concentrations of bilirubin in venous blood and in the lymph coming from the liver were measured at regular intervals throughout the experiments. Cholecystectomy had been performed in these experiments at the time of insertion of the lymphatic catheter.

General Description of the Response

A sharp increase in the bilirubin content of the lymph coming from the liver was observed within one to five hours in all six animals studied (Fig. 6). This reached a peak of from 13.5 mg. per cent. to 27.0 mg. per cent. in four to twelve hours. Thereafter it fell rapidly and by twenty-four hours it was found to be little higher than the venous blood concentration at that time. In the meantime, since obstruction had been produced, the concentration of bilirubin in the venous blood had risen slowly and more or less uniformly to level off at 24 hours, slightly below the lymphatic concentration /

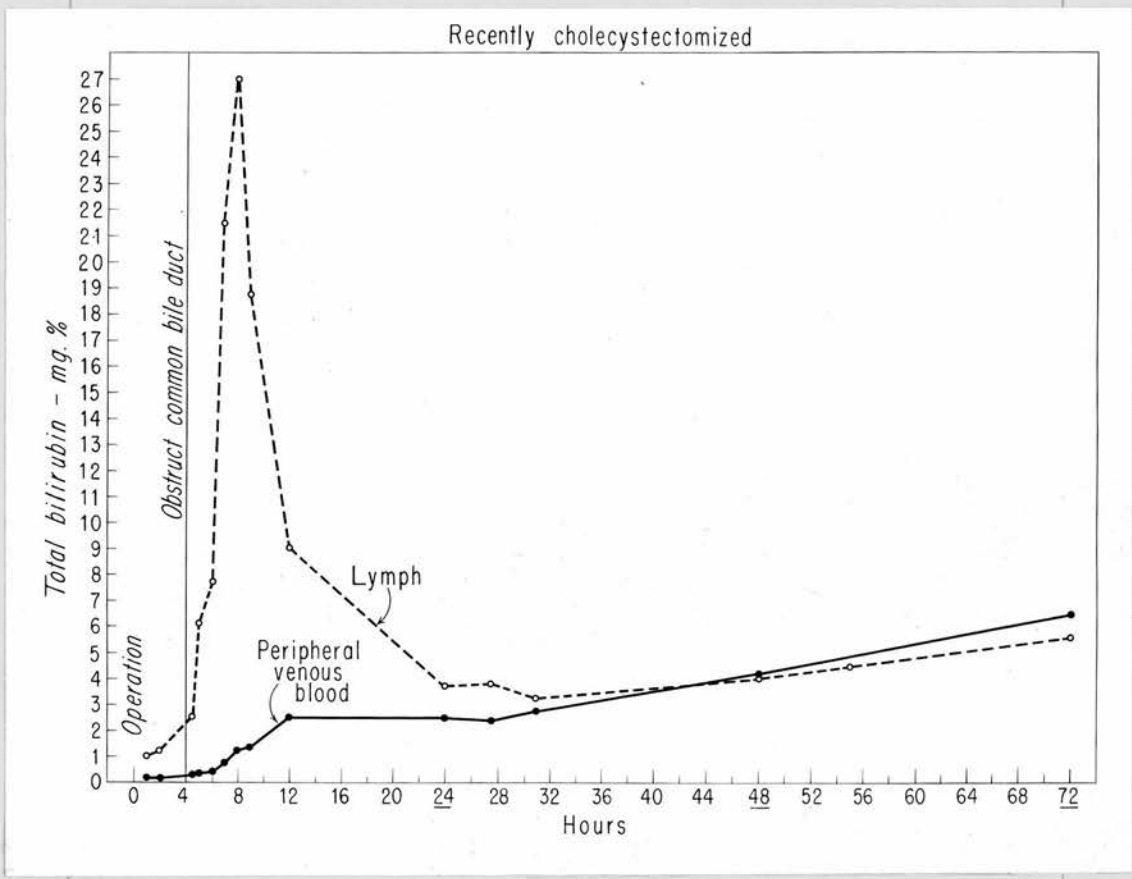


Fig. 6. **Acute Obstruction of the Common Bile Duct in the Recently Cholecystectomised Animal**

This figure depicts the findings in Experiment 1. A one hour test period of ether anaesthesia during the second day had no significant effect and is omitted for the sake of clarity.

tration, at between 2.5 mg. per cent. to 5.0 mg. per cent. During the next twenty-four hours no appreciable change occurred in the concentration of bilirubin from either source, but near the end of that time the blood concentration overtook that of the lymph and then began a slow increase in the bilirubin content of both media, the blood always remaining at a slightly higher level. This relationship was maintained up to ninety-two hours of obstruction which was the longest period of study.

In these six animals obstruction had been produced on the first, second or third day after operation and this by ligation and division of the common bile duct or by obstruction of a tube inserted into it, or by 'snaring' of the duct, or by injection of vinyl acetate into its lumen at the close of the operative procedure. It seemed therefore that this response was independent of the time after operation at which obstruction had been produced or of the method whereby this had been effected.

Detailed Description of the Experiments

Experiment 1 (Dog No. 1) (Fig. 6)

In this animal the gall bladder was removed, a Transflex tube was tied into the common bile duct and brought out through /

through the parietes and a similar tube was inserted into the liver lymphatic trunk and exteriorised in the same way. The common bile duct tube was obstructed with a metal nail four hours after the end of the operation. After a further period of four hours the concentration of bilirubin in the lymph coming from the liver had reached 27.0 mg. per cent. while that of the blood had risen to 1.2 mg. per cent. Thereafter the level of the lymph fell rapidly and after twenty-four hours of obstruction it contained 3.82 mg. per cent. while the blood concentration had risen to 2.37 mg. per cent. At this stage ether anaesthesia was induced and maintained for one hour. This had no appreciable effect on either level.

During the next eighteen hours little change occurred but after forty-four hours of obstruction the blood was found to contain 4.25 mg. per cent. of bilirubin while the concentration in the lymph was 4.12 mg. per cent. Thereafter the blood level remained higher until the end of the experiment at sixty-eight hours after obstruction had been produced.

Experiment 2 (Dog No. 2)

Here, in a preparation similar to that used in experiment 1, the common bile duct tube was obstructed two and a half hours after the end of the operation. Three hours later /

later the concentration of bilirubin in the lymph coming from the liver was found to be 13.5 mg. per cent. and that of the venous blood 0.75 mg. per cent. However, after twenty-one hours of obstruction, the lymph contained 3.62 mg. per cent. and the blood 3.0 mg. per cent. During the next twenty-four hours little change occurred, but after forty-nine hours of obstruction the blood contained 3.75 mg. per cent. while the concentration in the lymph had fallen to 3.0 mg. per cent. Thereafter the blood level remained higher until the end of the experiment at sixty-nine and a half hours after obstruction had been produced.

Experiment 3 (Dog No. 3)

This preparation differed from those above in that double ligation and division of the common bile duct was carried out at operation. Here also the concentration of bilirubin in the lymph rose rapidly. After five hours it contained 11.0 mg. per cent. and at nine hours 13.4 mg. per cent.

After twenty-four hours of obstruction however, it had fallen to 7.5 mg. per cent. while the blood concentration was 5.0 mg. per cent. At twenty-nine hours the levels were approximately equal and remained so although falling slightly until /

until fifty-six hours, at which timethe blood concentration was found to be 4.12 mg. per cent. and that of the lymph 3.8 mg. per cent. Thereafter and to the end of the experiment at ninety-two hours after obstruction had been produced, the concentration of bilirubin in the blood exceeded that in the lymph.

Experiment 4 (Dog No. 4)

In this preparation a braided silk snare was passed round the common bile duct as described under operative procedures. It was used to produce obstruction of the common bile duct twenty-five hours after the end of the operation, at which time the animal seemed fit and had apparently recovered satisfactorily from the anaesthesia. After two hours of obstruction the lymph was found to contain 21.0 mg. per cent. of bilirubin while the blood concentration was 0.27 mg. per cent. Thereafter the lymph concentration fell until, at twenty-three hours after obstruction, it was found to contain 3.5 mg. per cent. and the blood level at that time was 2.5 mg. per cent. Five hours later both were found to contain 2.5 mg. per cent. The lymph catheter was then found to be blocked and no further samples were obtained. It was noted however that for the next twenty-four hours the blood level /

level of bilirubin followed the pattern described in the preceding experiments in this group.

Experiment 5 (Dog No. 5)

This preparation was similar to that used in experiments 1 and 2. Here, however, the common bile duct tube was obstructed on the third post-operative day at fifty-two hours after the end of the operation. Within five hours the concentration of bilirubin in the lymph had risen to 11.12 mg. per cent. while that in the blood was found to be 2.5 mg. per cent.

At twenty-four hours after obstruction however on the fourth day the blood level was found to be 5.0 mg. per cent. while that of the lymph measured 4.62 mg. per cent.

Experiment 6 (Dog No. 6)

After cholecystectomy and canulation of the liver lymphatic trunk 6 ml. of vinyl acetate solution was injected into the common bile duct above a ligature placed in its distal half. At four hours after this injection the concentration of bilirubin in the lymph coming from the liver was found to be 11.5 mg. per cent. At twelve hours it had reached 17.5 mg. per cent. while that of the blood measured 1.25 mg. per cent. /

cent.

Twenty-four hours after the injection it had fallen to 7.25 mg. per cent. and the simultaneous blood sample was found to contain 3.0 mg. per cent.

ACUTE OBSTRUCTION OF THE COMMON BILE DUCT IN THE PREVIOUSLY CHOLECYSTECTOMISED ANIMAL

Acute obstruction of the common bile duct was produced in various ways in six animals (Nos. 7 to 12) and the concentrations of bilirubin in venous blood and in the lymph coming from the liver were measured at regular intervals throughout the experiments. Cholecystectomy had been performed three to four weeks before the second operation for insertion of the lymphatic catheter and obstruction of the biliary tree.

General Description of the Response

This was similar to that obtained in the recently cholecystectomised animal with certain exceptions. In general, the peak levels attained in the lymph were lower, ranging from 5.12 mg. per cent. to 18.75 mg. per cent. and the level at /

at which both blood and lymph concentrations first came together, after twenty-four hours of obstruction, were usually higher the blood levels ranging from 3.95 mg. per cent. to 6.75 mg. per cent. (Fig. 7). In three animals (Nos. 8, 10, 11) in which studies extending beyond the first twenty-four hours after obstruction were obtained, it was also noted that the blood levels now overtook those in the lymph and this relationship was maintained thenceforth. In contrast to the recently cholecystectomised group in which that period from twenty-four to forty-eight hours after obstruction was characterised by slightly higher lymph levels throughout.

Detailed Description of the Experiments

Experiment 7 (Dog No. 7) (Fig. 7)

The gall bladder had been removed from this animal some weeks previously. At a second operation a Transflex tube was tied into the common bile duct and brought out through the parietes and a similar tube was inserted into the liver lymphatic trunk and exteriorised. The common bile duct tube was obstructed with a metal nail four hours after the end of the operation. Within two hours the concentration of bili-

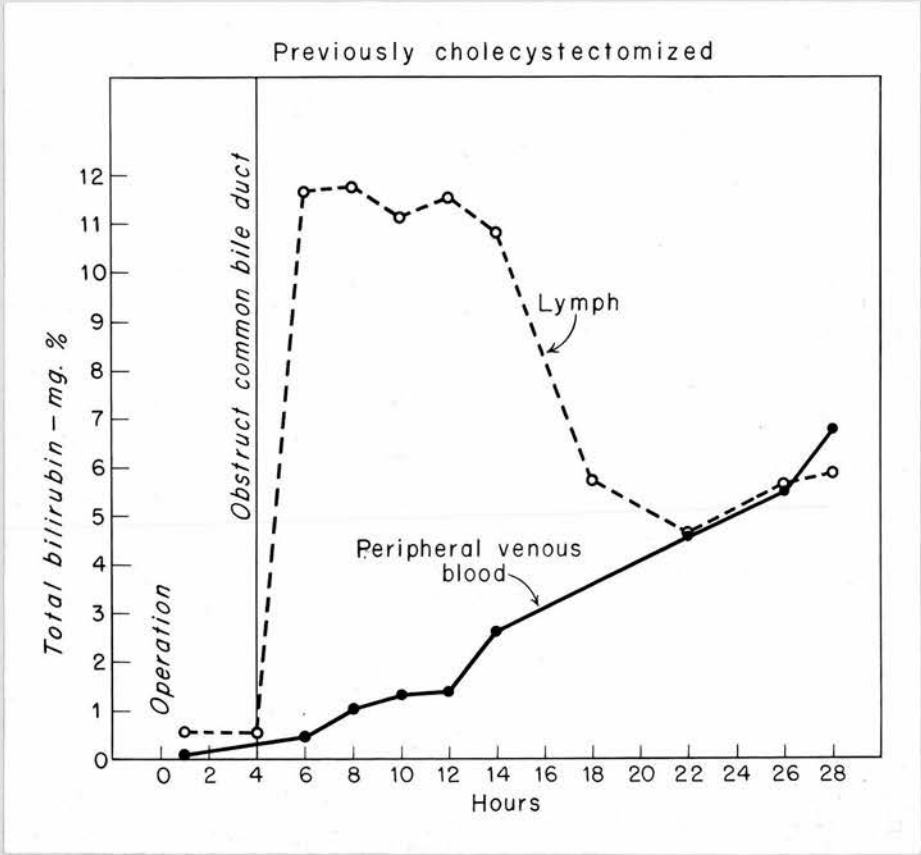


Fig. 7. **Acute Obstruction of the Common Bile Duct in the Previously Cholecystectomised Animal**

This figure depicts the findings in Experiment 7.

rubin in the lymph coming from the liver was found to be 11.62 mg. per cent. while that of the simultaneous blood sample was 0.45 mg. per cent. There was little change in the lymphatic concentration during the next eight hours and thereafter it fell rapidly so that at eighteen hours it was found to be 4.64 mg. per cent. while the blood concentration was 4.57 mg. per cent. After twenty-four hours of obstruction the blood level measured 6.75 mg. per cent. and that of the lymph 5.87 mg. per cent.

Experiment 8 (Dog No. 8)

This preparation differed from the above in that double ligation and division of the common bile duct was carried out at the second operation. Four hours later the concentration of bilirubin in the lymph coming from the liver was 4.87 mg. per cent. while the corresponding venous blood sample contained 0.6 mg. per cent. After seven hours of obstruction the lymph level rose to its peak of 5.12 mg. per cent. and then fell so that at twenty-four hours it contained 3.35 mg. per cent. while the blood concentration was found to be 3.95 mg. per cent.

For the next twelve hours little change occurred. The blood concentration remained slightly higher and then a gradual /

gradual increase began so that after forty-eight hours of obstruction the blood concentration was 5.75 mg. per cent. and that of the lymph 5.05 mg. per cent.

Experiment 9 (Dog No. 9)

In this preparation a braided silk snare was passed round the common bile duct at the second operation by the method described under operative procedures. It was used to produce obstruction of the common bile duct four hours after the end of that operation. Within seven and a half hours a peak level of 16.0 mg. per cent. was obtained in the lymph, while at this time the concentration of bilirubin in the venous blood was 1.12 mg. per cent. After ten hours the lymph concentration had fallen to 14.0 mg. per cent. and thereafter the lymph catheter ceased to function. It was noted however that after twenty hours of obstruction the blood concentration of bilirubin had risen to 5.5 mg. per cent.

Experiment 10 (Dog No. 10)

This preparation was similar to that used in experiment 8 in that the common bile duct was doubly tied and ligated at the second operation. Within ten hours the concentration of bilirubin in the lymph coming from the liver had reached its /

its peak level of 7.05 mg. per cent. while the corresponding venous blood sample contained 1.02 mg. per cent.

At twenty-four hours the lymph contained 5.05 mg. per cent. and the blood concentration had risen to 5.4 mg. per cent. During the next six hours no appreciable change occurred.

Experiment 11 (Dog No. 11)

This preparation was similar to that used in experiments 8 and 10 in that double ligation and division of the common bile duct was carried out at the time of the second operation. At ten hours the concentration of bilirubin in the lymph coming from the liver had reached its peak level of 18.75 mg. per cent. while the corresponding venous blood sample was found to contain 0.62 mg. per cent.

After twenty-four hours of obstruction the blood level had risen to 5.5 mg. per cent. exceeding that of the lymph which had fallen to 4.8 mg. per cent. This relationship was maintained to the end of the experiment at thirty-six hours at which time the levels were - blood 6.25 mg. per cent. and lymph 5.25 mg. per cent.

Experiment 12 (Dog No. 12)

This preparation was similar to that used in experiment

9 /

9 in that a braided silk snare was passed round the common bile duct at the second operation. It was used to produce obstruction of the duct twenty-five hours after the end of that operation. Within four hours the concentration of bilirubin in the lymph coming from the liver had risen to 6.25 mg. per cent. while the corresponding venous blood sample was found to contain 1.25 mg. per cent. The lymphatic catheter then became blocked and no further samples were obtained. It was noted however that after twenty-four hours of obstruction the blood concentration of bilirubin had risen to 4.12 mg. per cent., at forty-eight hours to 4.62 mg. per cent., and at seventy-two hours to 5.5 mg. per cent.

ACUTE OBSTRUCTION OF THE COMMON BILE DUCT WITH THE GALL BLADDER IN SITU

Acute obstruction of the common bile duct was produced in thirteen animals (Nos. 13 to 25) and the concentrations of bilirubin in venous blood and in the lymph coming from the liver were measured at regular intervals throughout the experiments. The gall bladder was examined at operation to ensure as far as possible that it was healthy and that it emptied /

emptied satisfactorily. It was left in situ. In the first seven preparations (Nos. 13 to 19) the lymphatic canula was inserted in the normal way and the course of the obstruction followed as in the previous groups. To extend the period of study however ligation and division of the common bile duct was carried out in six of these animals (Nos. 20 to 25) which were then left for periods of up to three days before "delayed" insertion of the lymphatic canula was carried out at a second operation. In this way it was possible to observe the course of an obstruction of three or four days duration with more certainty since, in the previous type of preparation, blockage of the lymphatic catheter had not infrequently occurred by this time.

General Description of the Response

During the first twenty-four hours of obstruction the presence of the gall bladder completely abolished the response observed in the recently and previously cholecystectomised groups (Fig. 8). There was no accumulation of bilirubin in the lymph coming from the liver and blood and lymph concentrations remained more or less at their pre-obstructive levels for two or three days, after which a gradual increase began, the /

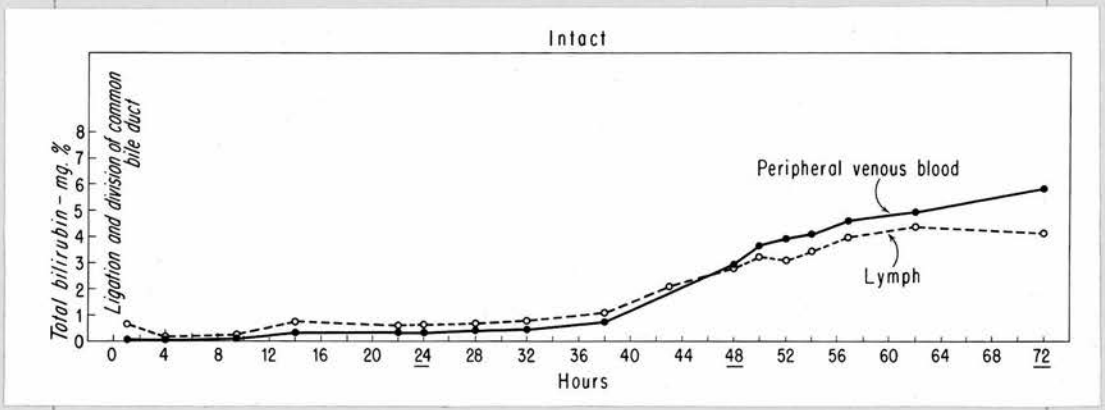


Fig. 8. **Acute Obstruction of the Common Bile Duct in the Animal with its Gall Bladder in situ.**

This figure depicts the findings in Experiment 13.

the blood level becoming and remaining the higher.

Detailed Description of the Experiments

Experiment 13 (Dog No. 13) (Fig. 8)

In this animal double ligation and division of the common bile duct was carried out and a Transflex tube was inserted into the liver lymphatic trunk. During the first thirty-eight hours there was little change in the concentration of bilirubin in lymph or blood. Both remained throughout below 1.0 mg. per cent., the lymph being slightly higher than the blood. Thereafter a gradual rise occurred the blood level overtaking that of the lymph so that after forty-eight hours of obstruction the blood was found to contain 2.95 mg. per cent. and the lymph 2.8 mg. per cent. The rise progressed and after seventy-two hours, at the end of the experiment, the blood contained 5.9 mg. per cent. and the lymph 4.2 mg. per cent.

Experiment 14 (Dog No. 14)

This preparation was similar to that used in experiment 13. During the thirty-one hours for which the lymphatic fistula continued to function the concentration of bilirubin in lymph and blood showed no change and at the end of this time /

time the lymph was found to contain 0.37 mg. per cent. while the blood contained 0.12 mg. per cent.

Experiment 15 (Dog No. 15)

This preparation was similar to numbers 13 and 14. The lymphatic fistula functioned for twenty-four hours only. No change in the concentration of bilirubin in either lymph or blood occurred during this time. At the end of the experiment the lymph was found to contain 0.42 mg. per cent. while the blood contained 0.27 mg. per cent.

Experiment 16 (Dog No. 16)

This preparation differed from those preceding it in this group in that a braided silk snare was placed round the common bile duct. This was used to produce obstruction of the biliary tree five hours after the end of the operation. During the next twenty-seven hours no significant change occurred in the concentration of bilirubin in either lymph or blood, the final concentrations at that time being - lymph 1.4 mg. per cent. and blood 0.75 mg. per cent.

Experiment 17 (Dog No. 17)

This preparation was similar to that used in experiment 16. The common bile duct was obstructed with a snare seven hours /

hours after operation. The lymphatic fistula functioned for nine hours only subsequent to this. During this time no significant change occurred in the concentration of bilirubin in either lymph or blood and the levels remained within the range of the previous experiments in this group.

Experiment 18 (Dog No. 18)

This preparation was similar to the last two. The common bile duct was obstructed with a snare five hours after the end of the operation. The lymphatic fistula functioned for seven hours only. During this time the response was similar to that described in the preceding experiments in this group.

Experiment 19 (Dog No. 19)

Here again, in a preparation similar to the last three, the common bile duct was obstructed with a snare twenty-nine hours after the end of the operation. The lymphatic fistula became blocked soon afterwards and no further samples were obtained. The venous blood concentration of bilirubin was followed for the next forty-eight hours and rose gradually to a level of 1.44 mg. per cent. at the end of this time.

Experiment 20 /

Experiment 20 (Dog No. 20)

In this preparation in an attempt to extend the duration of the study a technique of "delayed" canulation of the liver lymphatic trunk was employed. The common bile duct was doubly tied and divided at the first operation and twenty-four hours later the concentration of bilirubin in venous blood was found to be 0.6 mg. per cent. Canulation of the liver lymphatic trunk was then carried out. Twenty-four hours later little change had occurred in the bilirubin levels the lymph containing 1.2 mg. per cent. and the blood 0.98 mg. per cent. Within the next twenty-two hours however a slow rise began the blood concentration overtaking that of the lymph. At the end of the experiment, after seventy hours of biliary obstruction, the blood level was found to be 5.58 mg. per cent. while the lymph contained 5.29 mg. per cent. of bilirubin.

Experiment 21 (Dog No. 21)

This preparation was similar to that used in experiment 20. Here however canulation of the liver lymphatic trunk was delayed for forty-eight hours after obstruction had been produced. The concentration of bilirubin in the venous blood /

blood before the start of the operation was 2.25 mg. per cent. Little change occurred in either level during the next forty-eight hours and the final levels, after ninety-six hours of obstruction, were found to be - lymph 1.62 mg. per cent. and blood 1.5 mg. per cent.

Experiment 22 (Dog No. 22)

Here the procedure was similar to that used in experiment 20 where lymphatic canulation was delayed until twenty-four hours after obstruction had been produced. During the first fourteen hours after lymphatic canulation little change occurred, but eight hours later, that is after forty-six hours of obstruction, the concentration of bilirubin in the lymph coming from the liver was found to be 5.25 mg. per cent. while the blood contained 2.08 mg. per cent. During the next twelve hours the lymph concentration fell to near blood level and then began a gradual rise in both levels until at the end of the experiment, after seventy-two hours of biliary obstruction, the levels were - blood 5.25 mg. per cent. and lymph 4.9 mg. per cent.

Experiment 23 /

Experiment 23 (Dog No. 23)

This preparation was similar to that used in experiments 20 and 22 in that canulation of the lymphatic trunk was delayed for twenty-four hours after the production of biliary obstruction. The fistula functioned for twenty-eight hours during which time little change occurred in either concentration and the final levels, after fifty-four hours of obstruction, were - lymph 1.85 mg. per cent. and blood 0.85 mg. per cent.

Experiment 24 (Dog No. 24)

This preparation was similar to that used in experiment 21 in that lymphatic canulation was delayed until biliary obstruction had been present for forty-eight hours. The lymphatic fistula functioned for six hours only and at the end of this time the last sample of lymph contained 1.92 mg. per cent. of bilirubin and that of the blood 1.24 mg. per cent.

Experiment 25 (Dog No. 25)

Here canulation of the liver lymphatic trunk was delayed until seventy-two hours had elapsed from the time of obstruction of the biliary tree. The concentration of bilirubin in the venous blood at the end of the operation was found to be

4.37 /

4.37 mg. per cent. while the lymph level measured 3.62 mg. per cent. A slight rise took place in both levels during the next twenty-four hours so that at the end of the experiment, after ninety-six hours of biliary obstruction, the blood contained 5.16 mg. per cent. and the lymph 4.49 mg. per cent. of bilirubin.

Note on Some Additional Observations

A scrutiny of the protocols of the experiments in this group will show that slightly higher levels of bilirubin were measured in the lymph immediately after operation in dogs in which no biliary obstruction had, as yet, been produced. This rise was a transient one. A similar, more marked, but still transient rise was observed following 'delayed' liver lymphatic canulation in the presence of an already established biliary obstruction. This phenomenon seemed to be related to the effects of anaesthesia and operation themselves and not to biliary obstruction. A similar finding is reported by Bollman (1950). Takane (1932) has shown that anaesthesia reduces bile secretion. In experiment 22 an unusually high concentration of bilirubin was measured in the lymph coming from the liver for some hours during the second day of biliary obstruction. This will be discussed below (p. 158).

RECURRENT ACUTE OBSTRUCTION OF THE COMMON BILE DUCT IN THE CHOLECYSTECTOMISED ANIMAL

It was the purpose of this study to observe the effect of a second period of acute obstruction on the levels of bilirubin in the venous blood and in the lymph coming from the liver. In five cholecystectomised animals (Nos, 26 to 30) the common bile duct was obstructed for three hours on the day of operation and then released. On the second day the obstruction was renewed and maintained until the end of the experiment

General Description of the Response

The first bout of obstruction of three hours duration gave bilirubin levels in the lymph which rose rapidly and followed the pattern described above under the heading 'Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal'. Relief of obstruction was followed by a rapid fall to pre-obstructive levels. The blood concentration was not significantly affected during this three hour period. On the resumption /

resumption of obstruction a day later no sharp rise in the concentration of bilirubin in the lymph was obsdrved but a slow increase commenced. The concentration of bilirubin in the venous blood followed suit and after about twenty-four hours overtook that of the lymph and remained at a higher level thenceforth.

Detailed Description of the Experiments

Experiment 26 (Dog No. 26)

From this preparation the gall bladder was removed and a polyvinyl tube was tied into the common bile duct and brought out through the parietes in the right loin. Blood samples were withdrawn from a similar tube inserted into the splenic vein and advanced until its tip lay at the origin of the portal vein. The liver lymphatic trunk was canulated. Seventy-five minutes after the end of the operation the concentration of bilirubin in the lymph coming from the liver was found to be 0.25 mg. per cent. while the venous blood contained 0.17 mg. per cent. Three hours after the end of the operation the common bile duct tube was obstructed by a metal nail. Within a further three hours the concentration of bilirubin in the lymph had risen to 2.35 mg. per cent. while /

while that of the blood was found to be 0.22 mg. per cent. The common bile duct tube was now released and an hour later both had fallen to their pre-obstructive levels. Next morning little change had occurred and the common bile duct tube was obstructed for a second time. During the next twenty-four hours no significant change occurred, but after thirty-six hours the level of bilirubin in both lymph and blood had risen to 1.37 mg. per cent. Thereafter a slow rise began, the blood concentration overtaking that of the lymph and at the end of the experiment after fifty-two hours of obstruction the blood level had reached 2.85 mg. per cent. while the lymph contained 2.35 mg. per cent.

Experiment 27 (Dog No. 27)

This preparation was identical to that used in the previous experiment. The response was similar and after three hours of obstruction the concentration of bilirubin in the lymph coming from the liver had reached 8.02 mg. per cent. The obstruction was then relieved and the lymph concentration fell rapidly. Next day both blood and lymph levels were found to be lower than before obstruction. The common bile duct tube was now obstructed for the second time. Twenty hours later the lymph had risen to 1.20 mg. per cent. while the /

the blood contained 1.10 mg. per cent. The blood level now overtook that of the lymph and this slow rise continued so that at the end of the experiment, forty-six hours after obstruction had been repeated, the bilirubin concentration in the blood was found to be 3.62 mg. per cent. while the lymph contained 2.50 mg. per cent.

Experiment 28 (Dog No. 28)

This preparation was similar to both preceding it except that the blood samples were withdrawn from a tube inserted into a leg vein and pushed up into the iliac region. The response was similar to that described in the two previous experiments and at the end of the experiment, forty-eight hours having elapsed since the second obstruction was begun, the concentration of bilirubin in the venous blood was found to be 3.98 mg. per cent. while the lymph contained 3.75 mg. per cent.

Experiment 29 (Dog No. 29)

This preparation was similar to that used in the previous experiment. B.S.P. was given intravenously during the latter part of the first period of obstruction (see B.S.P. studies /

studies in 'Acute Obstruction of the Common Bile Duct'). It appeared to inhibit the passage of bilirubin into the lymph under these circumstances. This phenomenon was studied further and will be referred to later under the heading 'The Handling of B.S.P. by the Liver During Established Biliary Obstruction'.

The usual rise in the concentration of bilirubin in the lymph followed the first obstruction. Release of the obstruction produced the usual fall and reabsorption on the next day, the expected slow rise with the blood concentration gradually overtaking that of the lymph so that twenty-four hours after the second obstruction had been produced the concentration of bilirubin in the venous blood measured 2.5 mg. per cent. while that of the lymph was found to be 1.8 mg. per cent.

Experiment 30 (Dog No. 30)

This experiment resembled the previous one and was of the nature of a combined study. Here also B.S.P. injection was commenced two hours after the first obstruction had been produced (see 'B.S.P. Studies in Acute Obstruction'). Bilirubin results conformed to the pattern described in the preceding experiments in this group but here again there seemed to be inhibition of the passage of bilirubin into the lymph consequent upon the B.S.P. injection. This will be discussed below (page 138).

CHRONIC OBSTRUCTION OF THE COMMON BILE DUCT IN THE CHOLECYSTECTOMISED ANIMAL

Double ligation and division of the common bile duct was carried out in five cholecystectomised animals (Nos. 26 to 30). After an interval varying from six days to five weeks 'delayed' canulation of the liver lymphatic trunk was carried out and the concentrations of bilirubin in venous blood and in the lymph coming from the liver were measured at regular intervals throughout the experiments.

General Description of the Response

The concentrations of bilirubin in the systemic venous blood of these animals were uniformly and consistently higher than those found in the corresponding samples of liver lymph.

Detailed Description of the Experiments

Experiment 31 (Dog No. 31)

Double ligation and division of the common bile duct was carried out and the gall bladder was removed. The animal was returned to the kennels and six days later 'delayed' canulation /

ion of the liver lymphatic trunk was performed at a second operation. One hour after the end of the operation, by which timethe animal had recovered from the anaesthetic, the concentration of bilirubin in the venous blood was found to be 6.5 mg. per cent. while the lymph level measured 3.64 mg. per cent. Little change occurred during the next fifty-five hours and the final levels at that time were found to be - blood 6.37 mg. per cent. and lymph 5.01 mg. per cent.

Experiment 32 (Dog No. 32)

Double ligation and division of the common bile duct was carried out and the gall bladder was removed. The animal was returned to the kennels and three weeks later 'delayed' canulation of the liver lymphatic trunk was performed at a second operation. One hour after the end of this operation by which time the animal had recovered from the anaesthetic the concentration of bilirubin in the venous blood was found to be 8.25 mg. per cent. while the lymph level measured 4.5 mg. per cent. During the next twenty-four hours little change occurred and the final samples at that time were found to be - blood 7.5 mg. per cent. and lymph 4.5 mg. per cent.

Experiment 33 /

Experiment 33 (Dog No. 33)

This preparation was similar to that in experiments 26 and 27. Five weeks after obstruction had been produced the liver lymphatic trunk was canulated. The concentration of bilirubin in the blood one hour after the end of the operation was found to be 21.2 mg. per cent. while the corresponding sample of liver lymph was found to contain 10.56mg. per cent. During the next twenty-four hours the gap between these totals narrowed and at the end of this time the blood contained 12.0 mg. per cent. and the lymph 8.0 mg. per cent.

Experiment 34 (Dog No. 34)

This preparation was similar to those preceding it in this group. After twelve days 'delayed' canulation of the liver lymphatic trunk was carried out. A specimen of lymph obtained during the operation contained 5.62 mg. per cent. while a simultaneous sample of the inferior cavel venous blood contained 8.25 mg. per cent. The animal's condition was poor and it was sacrificed under the anaesthetic.

Experiment 35 (Dog No. 35)

This preparation was similar to those preceding it in this group. After nineteen days 'delayed' canulation of the liver /

liver lymphatic trunk was carried out. A specimen of lymph obtained during the operation contained 22.25 mg. per cent. while a simultaneous sample of blood from the inferior vena cava, below the liver, was found to contain 27.5 mg. per cent. The animal's condition was poor and it was therefore sacrificed before it recovered from the anaesthetic.

CHRONIC OBSTRUCTION OF THE COMMON BILE DUCT WITH THE GALL BLADDER IN SITU

Double ligation and division of the common bile duct was carried out in three animals (Nos. 31 to 33) and the gall bladder was left undisturbed. After an interval varying from four days to thirteen days 'delayed' canulation of the liver lymphatic trunk was carried out and the concentrations of bilirubin in the venous blood and in the lymph coming from the liver were measured at regular intervals throughout the experiments.

General Description of the Response

Here also the concentrations of bilirubin in the venous blood /

blood of these animals were uniformly and consistently higher than those found in the corresponding samples of liver lymph.

Detailed Description of the Experiments

Experiment 36 (Dog No. 36)

Double ligation and division of the common bile duct was carried out and the gall bladder was left undisturbed. The animal was returned to the kennels and eight days later 'delayed' canulation of the liver lymphatic trunk was carried out. One hour after the end of the operation the concentration of bilirubin in the venous blood was found to be 8.37 mg. per cent. while the corresponding lymph sample contained 5.25 mg. per cent. Shortly afterwards due to a fault in handling the lymph catheter was displaced. It was noticed however that twenty-four hours post-operatively the concentration of bilirubin in the venous blood was 8.87 mg. per cent. The animal's condition was good and it was therefore returned to the kennels. Five days later the lymphatic trunk was again canulated and a sample withdrawn, under anaesthesia, was found to contain 5.8 mg. per cent. while a corresponding sample of blood from the inferior vena cava below the liver contained 8.0 mg. per cent. The animal was sacrificed before /

before it recovered from the anaesthetic.

Experiment 37 (Dog No. 37)

This preparation was similar to that used in experiment 31. Four days later 'delayed' canulation of the liver lymphatic trunk was carried out. One hour after the end of the operation the concentration of bilirubin in the venous blood of this animal was found to be 7.12 mg. per cent. while the corresponding sample of liver lymph contained 6.37 mg. per cent. Soon thereafter the lymphatic catheter became blocked.

Experiment 38 (Dog No. 38)

This preparation was similar to both those preceding it and biliary obstruction had been present for nine days before canulation of the liver lymphatic trunk was carried out. A specimen of lymph obtained at operation was found to contain 3.0 mg. per cent. of bilirubin while the corresponding sample of venous blood measured 3.5 mg. per cent. The condition of the animal was poor and it was sacrificed before recovering from the anaesthetic.

BROMSULPHTHALEIN

STUDIES

B.S.P. STUDIES

ACUTE, RECURRENT ACUTE AND CHRONIC OBSTRUCTION OF THE COMMON BILE DUCT IN THE CHOLECYSTECTOMISED ANIMAL

By means of a constant speed injection apparatus a continuous intravenous infusion of B.S.P. was given to nine cholecystectomised animals (Nos. 28, 29, 30, 39, 40, 41, 42, 43, 44). Because of the toxicity of the drug prolonged administrations of this nature have an adverse effect on the animal. With the dosages used however it was possible to continue the infusion for up to six hours without producing any apparent depression. The concentrations of B.S.P. in the venous blood and in the lymph coming from the liver were measured at regular intervals.

In six of these preparations (Nos. 29, 30, 39, 40, 41, 42) the bile was being collected from a tube tied into the common bile duct and brought out through the parietes. The tube was obstructed with a metal nail and the effect of this procedure on the levels of B.S.P. in the above media was observed.

The common bile duct was released after these experiments and the opportunity was taken to repeat them in three animals /

animals (Nos. 30, 39, 40) on the following day so that the effect of a second period of acute obstruction could be studied.

In the three remaining animals in this group (Nos. 28, 43, 44) biliary obstruction had been present for thirty-six hours, seven days and ten days respectively before the B.S.P. injection was begun.

General Description of the Response

In all six animals (Nos. 29, 30, 39, 40, 41, 42) in which acute obstruction of the common bile duct was produced, B.S.P. levels in the lymph coming from the liver were clearly and consistently higher than the corresponding venous blood levels. This was the case whether the B.S.P. injection was started before obstruction was present or during it.

The effect of a second period of acute obstruction was studied in three of these preparations (Nos. 30, 39, 40). Here the concentrations of B.S.P. found in the liver lymph were lower than those in the corresponding blood samples (Fig. 9).

When biliary obstruction had been present for periods of up to ten days studies on a further group of three animals (Nos. 28, 43, 44) showed that here also the concentrations of B.S.P. /

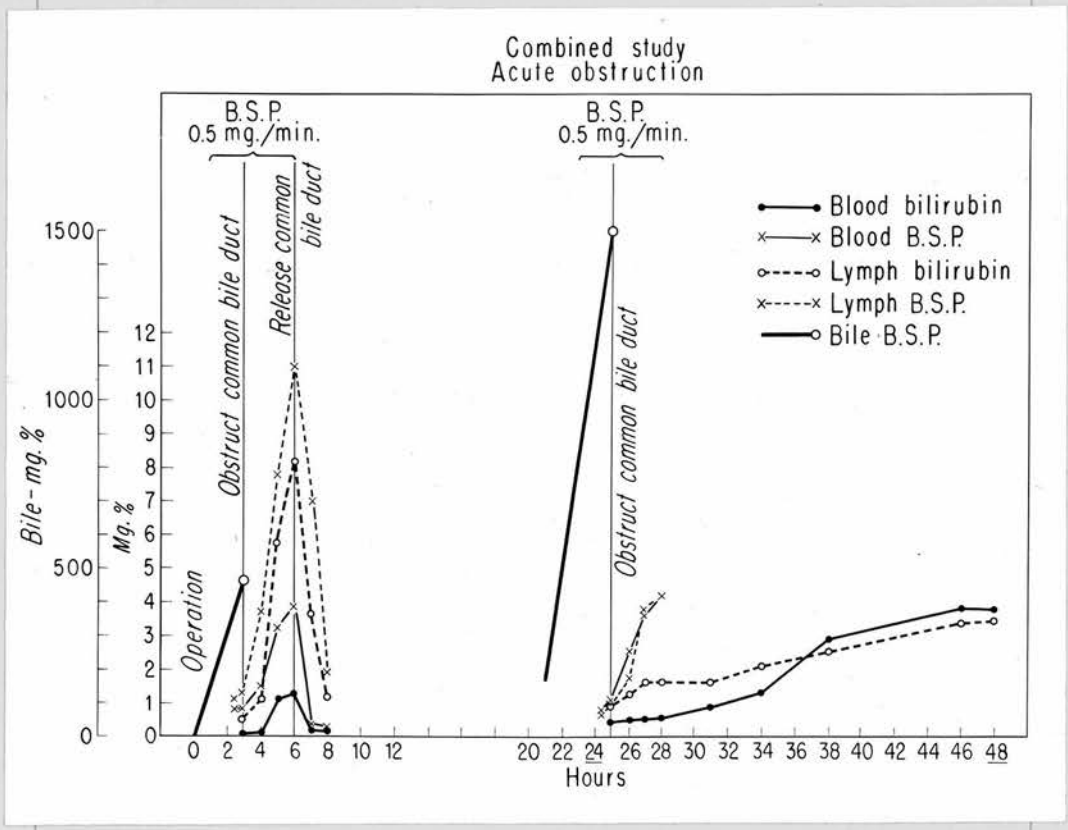


Fig. 9. **Recurrent Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal**

This figure depicts the findings in Experiment 40. It was of the nature of a combined study. The common bile duct was obstructed for a period of three hours on the first day and the effect of this procedure on the levels of bilirubin and B.S.P. in the liver lymph and venous blood was measured. The experiment was repeated next day but here the obstruction was maintained. 'Total bilirubin' measurements are shown.

B.S.P. in the liver lymph were lower than the venous blood levels (Fig.10)

Detailed Description of the Experiments

Experiment 39 (Dog No. 39)

From this preparation the gall bladder was removed, a Transflex tube was tied into the common bile duct and brought out through the parietes. The portal vein was canulated via the splenic vein and a Transflex tube was inserted into the liver lymphatic trunk and exteriorised. By means of a constant speed injection apparatus 1.0 mg. of B.S.P. was injected intravenously per minute. One hour later the concentration of B.S.P. in the liver lymph was found to be 1.22 mg. per cent. while the portal venous blood contained 0.80 mg. per cent. At this stage the bile contained 312.50 mg. per cent. Thirty minutes later the common bile duct tube was obstructed with a metal nail and during the next two hours the levels of B.S.P. in the lymph were clearly higher than those in the portal venous blood so that at the end of this time the lymph was found to contain 4.70 mg. per cent. while the blood level was 2.40 mg. per cent. The obstruction was now relieved.

Next morning the injection was recommenced. One hour later /

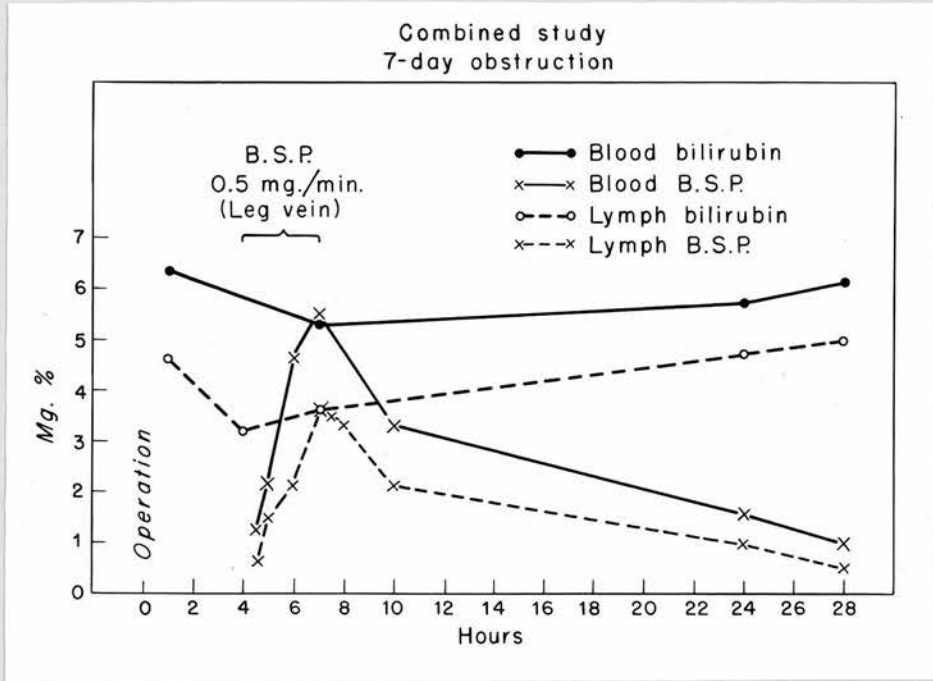


Fig. 10. **Chronic Obstruction of the Common Bile Duct in the Cholecystectomised Animal**

This figure depicts the findings in Experiment 43. It was of the nature of a combined study in which the levels of bilirubin and B.S.P. in the liver lymph and venous blood were measured. B.S.P. was given by a constant speed injection apparatus. 'Total bilirubin' measurements are shown.

later the lymph was found to contain 0.50 mg. per cent. of B.S.P. while the portal venous blood contained 1.2 mg. per cent. At this stage the bile again contained 312.50 mg. per cent. Thirty minutes later the obstruction was repeated but during the next three hours the levels of B.S.P. in the lymph were consistently lower than those in the portal blood. At the end of this timethe lymph contained 5.15 mg. per cent. and the portal blood 6.27 mg. per cent. of B.S.P.

Experiment 40 (Dog. No. 40) (Fig. 9.)

This preparation was similar to the previous one except that blood samples were withdrawn from a tube inserted into a leg vein and pushed up into the iliac region. It was used for a combined study in which both bilirubin and B.S.P. were measured in lymph and blood. The same procedure was followed but in this case 0.5 mg. of B.S.P. per minute were injected. Two hours later the concentration of B.S.P. in the liver lymph was found to be 1.25 mg. per cent. while that of the venous blood was 0.87 mg. per cent. At this stage the bile contained 465 mg. per cent. of B.S.P. The common bile duct tube was now obstructed and during the next three hours the concentrations of both bilirubin and B.S.P. in the lymph clearly exceeded those in the blood so that at the end of this time the lymph contained 8.12 mg. per cent. of bilirubin and /

and 11.0 mg. per cent. of B.S.P. while the blood contained 1.25 mg. per cent. of bilirubin and 3.87 mg. per cent. of B.S.P. The obstruction was now relieved. Next morning the injection was recommenced and two hours later the lymph was found to contain 0.92 mg. per cent. of B.S.P. while the venous blood level measured 1.0 mg. per cent. At this stage the concentration of B.S.P. in the bile was found to be 1,500 mg. per cent. The common bile duct tube was now obstructed for the second time and during the next three hours bilirubin levels showed little change. B.S.P. levels, although steadily increasing, were more or less equal. So that at the end of this time the liver lymph contained 1.65 mg. per cent. of bilirubin and 4.12 mg. per cent. of B.S.P. while the corresponding venous blood concentrations were found to be 0.52 mg. per cent. and 4.12 mg. per cent.

The B.S.P. injection was now stopped but bilirubin measurements were continued. After thirteen hours from the recommencement of obstruction the venous blood level of bilirubin overtook that of the lymph and this relationship was maintained during the next twelve hours so that after twenty-five hours the blood contained 3.75 mg. per cent. of bilirubin while the lymph level was 3.5 mg. per cent. (See 'Recurrent Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal', page 107).

Experiment 30 /

Experiment 30 (Dog No. 30)

This experiment was of the nature of a combined study and the preparation has been described in the bilirubin results reported in the chapter headed 'Recurrent Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal'. Only the B.S.P. findings will be summarised here.

The common bile duct tube was obstructed and two hours later an injection of 1.0 mg. per minute of B.S.P. was commenced. During the next three hours the concentrations of B.S.P. in the liver lymph were consistently higher than those in the venous blood. The injection was now stopped and the common bile duct tube released. The experiment was repeated next day but here the lymph levels of B.S.P. were clearly lower than those in the venous blood so that after three hours they measured - lymph 4.62 mg. per cent. and blood 6.75 mg. per cent.

Experiment 41 (Dog No. 41)

This preparation was similar to that used in experiment 40 and was also used for a combined study (Compare 'Acute Obstruction of the Common Bile Duct in the Recently Cholecystectomised Animal'). An injection of 0.5 mg. per minute of B.S.P. was given. One and a half hours later the concentration of B.S.P. which was found in the liver /

liver lymph was 2.0 mg. per cent. while the venous blood contained 0.87 mg. per cent. Thirty minutes later the bile concentration of B.S.P. was found to be 530 mg. per cent. and the common bile duct tube was then obstructed with a metal nail. During the next three hours the concentrations of bilirubin and B.S.P. in the liver lymph clearly exceeded those in the corresponding venous blood samples. So that at the end of this time the lymph was found to contain 6.3 mg. per cent. of bilirubin and 6.87 mg. per cent. of B.S.P. while venous blood levels were 0.38 mg. per gent. and 3.25 mg. per cent. respectively.

Experiment 29 (Dog No. 29)

This preparation was similar to that preceding it. It was of the nature of a combined study. The bilirubin results are reported under the headings 'Recurrent Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal' and also under 'The Handling of B.S.P. by the Liver During Established Biliary Obstruction'. Only the B.S.P. findings will be summarised here. The common bile duct tube was obstructed with a metal nail and two hours later a continuous injection of 1.0 mg. per minute of B.S.P. was begun. During the next two hours the levels of B.S.P. in the liver lymph exceeded /

exceeded those of the blood. At the end of that time the lymph contained 8.87 mg. per cent. of B.S.P. while the blood concentration was 5.87 mg. per cent.

Experiment 42 (Dog No. 42)

This preparation was similar to the above and was used for a combined study. The bilirubin findings are reported under the heading 'The Handling of B.S.P. by the Liver During Established Biliary Obstruction'. Only the B.S.P. findings will be summarised here. The common bile duct was obstructed and four hours later a continuous injection of 1.0 mg. per minute of B.S.P. was commenced. During the next three hours concentrations of B.S.P. in the liver lymph greatly exceeded those in the venous blood so that at the end of this time the lymph contained 29.5 mg. per cent. while the venous blood level was found to be 7.87 mg. per cent.

Experiment 28 (Dog No. 28)

This preparation was similar to the previous one. It was of the nature of a combined study. The bilirubin results are described under the heading 'Recurrent Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal'. Only the B.S.P. findings will be summarised here and /

and they will be referred to again in the chapter headed 'The Handling of B.S.P. by the Liver During Established Biliary Obstruction'.

The common bile duct was obstructed for the second time on the day after operation and about thirty-six hours later an injection of 1.0 mg. per minute of B.S.P. into a leg vein was commenced. The levels of B.S.P. in the liver lymph during the next three hours were clearly below those in the venous blood. At the end of this time the concentration of B.S.P. in the liver lymph had risen to 5.6 mg. per cent. while the venous blood level was 7.75 mg. per cent.

Experiment 43 (Dog No. 43) (Fig.10)

In this preparation the gall bladder was removed and double ligation and division of the common bile duct was carried out. The animal was returned to the kennels and seven days later, at a second operation, canulation of the liver lymphatic trunk was effected. A combined study was undertaken and the bilirubin findings followed the pattern described in the chapter headed 'Chronic Obstruction of the Common Bile Duct in the Cholecystectomised Animal', in that the concentration of bilirubin in the liver lymph was consistently lower than that found in the venous blood.

A /

A continuous injection of 0.5 mg. per minute of B.S.P. was made and during the next three hours B.S.P. levels in the liver lymph were clearly lower than those in the venous blood. At the end of this time the lymph contained 3.62 mg. per cent. and the blood 5.5 mg. per cent. of B.S.P.

Experiment 44 (Dog No. 44)

This preparation was similar to the previous one except that ten days were allowed to elapse before the second operation for insertion of a lymphatic canula was carried out. Here again a combined study was attempted and the bilirubin results conformed to the pattern described in the chapter headed 'Chronic Obstruction of the Common Bile Duct in the Cholecystectomised Animal'.

A continuous injection of 0.5 mg. per minute of B.S.P. was commenced. During the next three hours the concentrations of B.S.P. in the lymph coming from the liver were clearly lower than those found in the venous blood. At the end of this time the lymph contained 3.37 mg. per cent. and the blood 4.87 mg. per cent. of B.S.P.

THE RAT EXPERIMENTS

ACUTE AND CHRONIC OBSTRUCTION OF THE COMMON BILE DUCT

It seemed possible that the responses observed in the canine experiments might be confined to that species only. A second series of studies was therefore undertaken on the rat to investigate this. The rat was chosen as being the only other mammal readily available in which the creation of lymphatic fistulae and their maintenance after recovery from anaesthesia did not present unsurmountable difficulties.

Accordingly liver lymphatic fistulae were created in twelve rats (A to L) as described on page 68 in the chapter headed 'Methods'. These were divided into two groups A to F and G to L. In the first group (A to F) double ligation and division of the common bile duct was carried out at the time of insertion of the lymphatic catheter. The animal was then placed in a cage which limited its activity (Fig. 5.) and samples of lymph were collected at intervals for twenty-four hours (Rats A to C) or for forty-eight hours (Rats D to F). At the end of the experiment blood was obtained by direct cardiac puncture. It seemed undesirable to withdraw blood samples more frequently than /

than this. At least 2 ml. of blood are required to do the assay and even a few such venesections could produce a significant reduction of the animal's blood volume.

In the second group of experiments (G to L) double ligation and division of the common bile duct was carried out and the animals were returned to their cages. Canulation of the liver lymphatic trunk was carried out at a second operation two to ten days later. The concentrations of bilirubin in the samples of lymph and blood were now measured.

Results

No sharp rise in the concentration of bilirubin in the lymph coming from the liver, such as was observed in the canine experiments (pages 84,85), occurred in these preparations. Instead a progressive increase followed so that in animals A to C, after twenty-four hours of biliary obstruction, the lymph contained between 2 mg. per cent. and 3 mg. per cent. of bilirubin, as did the blood, but in each case the lymph level was slightly higher than that found in the blood.

In experiments D to F where obstruction had been present for forty-eight hours this steady increase in the concentrationsof bilirubin in the lymph coming from the liver continued.

At /

At the end of that time the lymph levels ranged from 5.12 mg. per cent. to 6.7 mg. per cent. while the blood levels, which were by now definitely higher, ranged from 5.75 mg. per cent. to 7.25 mg. per cent.

Where biliary obstruction had been present for up to ten days (G to L) the blood concentrations of bilirubin in five preparations were higher than those found in the liver lymph. In the remaining animal (I) the levels were equal.

STUDIES ON THE MECHANISM OF THE STANDARD RESPONSE

THE HANDLING OF B.S.P. BY THE LIVER DURING ESTABLISHED BILIARY OBSTRUCTION

In this study use was made of the fact that the liver seems to handle B.S.P. more or less in the same way as bilirubin (page 47).

There was evidence from the above studies on cholecystectomised animals that during the first twenty-four hours of biliary obstruction the liver might still be concentrating bilirubin since throughout part at least of this period lymph levels greatly exceeded those of the blood. It was not clear however whether the bilirubin found in the lymph had come from some pre-existing intra-hepatic source, such as the biliary tree, or whether it was in fact continuously being extracted from blood passing through the viscus concentrated and then diverted into the lymph. If indeed during this period a mechanism which concentrated bilirubin was still functioning it seemed reasonable to expect that the introduction of B.S.P. as a competitor into the system might impair its performance.

When however biliary obstruction had been present for twenty-four hours there was nothing to suggest that a concentrating /

concentrating mechanism was still active since the lymph levels of bilirubin at this stage were not significantly higher than those of the blood. It remained to be seen if the liver was now unable to concentrate B.S.P.

Accordingly B.S.P. was injected, as a 'Tracer' for bilirubin, into seven cholecystectomised animals (Nos. 29, 30, 42, 45,46, 47, 48). A constant speed injection apparatus was used and to one group (Nos. 29, 30, 42, 45) the drug was given a few hours after obstruction was produced. To a further group (Nos. 46, 47, 48) the administration was withheld until twenty-four hours had elapsed from the time when obstruction had been established. Bilirubin levels in liver lymph and venous blood were measured throughout each experiment in addition to the B.S.P. measurements.

General Description of the Response

In four animals (Nos. 29, 30, 42, 45) a continuous intravenous injection of B.S.P. in isotonic saline was commenced at two to four hours after obstruction had been produced. On each occasion it passed quickly into the lymph and was found there in concentrations which were clearly in excess of those in the corresponding venous blood samples (page 119) B.S.P. in Acute Obstruction')

It /

It was noticed however that this accumulation of B.S.P. in the lymph was accompanied by a reduction in the amount of bilirubin which should normally have passed into it at this stage of such an obstruction (page 84 . 'Acute obstruction in Recently Cholecystectomised Animals'). This effect was not obtained when saline alone was given (Nos. 46, 47) and was independent of the volume of saline used as the vehicle for infusion (Nos. 42, 45). In one of these experiments (No. 45) the injection was stopped but obstruction was maintained. This was followed by a secondary increase in the concentration of bilirubin appearing in the lymph (Fig11).

In a further group of animals (Nos. 46 to 48) a similar injection of B.S.P. was given twenty-four to twenty-eight hours after obstruction had been produced, by which time the concentration of bilirubin in the lymph had fallen to near the blood level (page 84, 'Acute Obstruction in Recently Cholecystectomised Animals'). Here however the concentration of B.S.P. in the lymph coming from the liver during the injection did not exceed that of the corresponding venous blood sample.

Detailed /

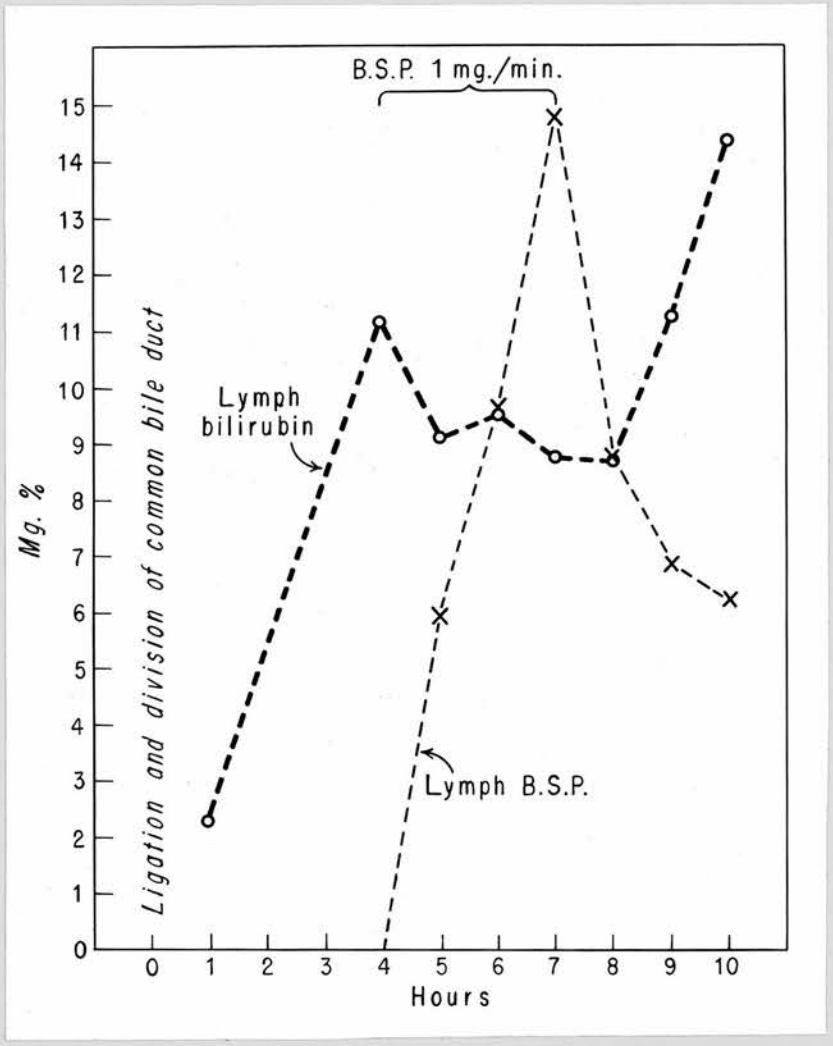


Fig. 11. **The Handling of B.S.P. by the Liver During Acute Obstruction of the Common Bile Duct in the Cholecystectomised Animal**

This figure depicts the findings in Experiment 45. 'Total bilirubin' measurements are shown.

Detailed Description of the Experiments

Experiment 45 (Dog No. 45) (Fig.11)

For this preparation the gall bladder was removed, the common bile duct was doubly tied and divided and a canula was inserted into the liver lymphatic trunk. Four hours later the concentration of bilirubin in the lymph coming from the liver was found to be 11.12 mg. per cent. At this stage a continuous injection of 1.0 mg. B.S.P. per minute was commenced. The dye was dissolved in isotonic saline, 40 ml. of which were given in each hour. One hour later the concentration of bilirubin had fallen to 9.12 mg. per cent. Little change occurred during the next two hours although all this time the B.S.P. content of the lymph was rising rapidly so that after three hours of injection it contained 14.75 mg. per cent. while the venous blood B.S.P. level was 7.0 mg. per cent. (page 119, 'B.S.P. in Acute Obstruction'). The injection was now stopped and a secondary increase in the bilirubin content of the lymph began. Three hours later this was found to be 14.37 mg. per cent.

Experiment 42 /

Experiment 42 (Dog No. 42)

This preparation is described on page 128 in the chapter headed 'B.S.P. Studies'. The B.S.P. findings during acute obstruction are reported there. Only the bilirubin data will be described here. Four hours after obstruction of the common bile duct the concentration of bilirubin in the lymph coming from the liver was found to be 7.5 mg. per cent. A continuous injection of B.S.P. was now started, 1.0 mg. per minute being given into a leg vein. This was dissolved in isotonic saline 5.0 ml. of which were given in each hour. The injection was followed by a decrease in the concentration of bilirubin in the lymph which contained 4.5 mg. per cent. after two hours.

Experiment 29 (Dog No. 29)

This preparation has been described on page 127. The common bile duct tube was obstructed and two hours later the concentration of bilirubin in the lymph coming from the liver was found to be 3.62 mg. per cent. A continuous injection of 1.0 mg. per minute of B.S.P. was commenced. This was dissolved in isotonic saline 40 ml. of which were given in each hour. Two hours later the lymph contained 2.75 mg. per cent. /

cent. of bilirubin. In the meantime the B.S.P. content of the lymph had been rising steadily so that at this stage it contained 8.87 mg. per cent. while the venous blood B.S.P. level was 5.87 mg. per cent. (compare page 119 'B.S.P. in Acute Obstruction').

Experiment 30 (Dog. No. 30)

The bilirubin and B.S.P. findings in this animal have already been described on pages 84 and 119 ('Bilirubin - Recurrent Acute Obstruction: B.S.P. - Acute Obstruction'). Here, after two hours of biliary obstruction the injection of 1.0 mg. per minute of B.S.P. dissolved in isotonic saline given at the rate of 40 ml. per hour seemed to halt the expected rise in the bilirubin content of the liver lymph. When the injection had lasted for three hours the bilirubin level in the liver lymph had risen from 5.5 mg. per cent. at the start of the injection to 6.75 mg. per cent.

In the meantime however the concentration of B.S.P. in the lymph had risen rapidly so that it now contained 8.25 mg. per cent. while the venous blood B.S.P. level was 6.62 mg. per cent. (see page 119 'B.S.P. in Acute Obstruction').

Experiment 46 /

Experiment 46 (Dog No. 46)

For this preparation the gall bladder was removed, a Transflex tube was tied into the common bile duct and brought out through the parietes along with a similar tube which had been inserted into the liver lymphatic trunk. The common bile duct tube was obstructed with a metal nail and four hours later the lymph contained 8.0 mg. per cent. of bilirubin. A saline control injection was now commenced, 40 ml. of isotonic saline being given each hour by a constant speed injection apparatus into a leg vein. The injection was continued for three hours. During this time the concentration of bilirubin in the lymph continued to rise rapidly and appeared to be unaffected by this infusion. At the end of this time it contained 19.6 mg. per cent. of bilirubin. Next morning it had fallen to 3.25 mg. per cent. and the blood level had risen to 2.87 mg. per cent. (see page 84 'Acute Obstruction in Recently Cholecystectomised Animals').

Twenty-eight hours after obstruction had been produced a continuous intravenous injection of 1.0 mg. per minute of B.S.P. was given in the same amount of isotonic saline as had been used during the first infusion in this experiment. The injection was continued for three hours. Throughout this time the concentration of B.S.P. in the lymph was clearly lower than the corresponding venous blood level. So that at the /

the end of the injection the lymph contained 6.0 mg. per cent. while the venous blood level was 8.75 mg. per cent.

Experiment 47 (Dog. No. 47)

This preparation was similar to that used in the preceding experiment and the procedure was the same. Here again after four hours of obstruction 40 ml. of isotonic saline were injected hourly for three hours. This failed to halt the increase in the concentration of bilirubin in the lymph coming from the liver which has been shown to occur during this type of obstruction (see page 84 'Acute Obstruction in the Recently Cholecystectomised Animals').

Next morning the concentration of bilirubin in the lymph had fallen to 5.32 mg. per cent. while that of the blood had risen to 4.62 mg. per cent. An intravenous injection of B.S.P., similar to that used in the previous experiment, was given during the next three hours and lymph levels of B.S.P. throughout this time remained clearly lower than those of the blood. So that at the end of the injection the lymph contained 10.0 mg. per cent. of B.S.P. and the blood 13.0 mg. per cent.

Experiment 48 /

Experiment 48 (Dog No. 48)

This preparation was similar to both preceding it. The common bile duct tube was obstructed with a metal nail and within six hours the lymph contained 17.8 mg. per cent. of bilirubin.

After twenty-eight hours of biliary obstruction the lymph contained 3.75 mg. per cent. of bilirubin while the blood level was 3.5 mg. per cent. A continuous injection of 1.0 mg. per minute of B.S.P. was now commenced. It was given for a period of three hours in isotonic saline, 40 ml. of which were delivered by the injection apparatus in each hour. Throughout this period the concentration of B.S.P. in the lymph coming from the liver was clearly lower than the corresponding venous blood level. So that at the end of this time it contained 8.25 mg. per cent. while the blood concentration was 11.0 mg. per cent.

CHROMATOGRAPHIC IDENTIFICATION OF THE DIRECT PIGMENTS PRESENT IN LIVER LYMPH DURING ACUTE BILIARY OBSTRUCTION

Scrutiny of the protocols of experiments Numbers 1 to 12 will show that during the first twenty-four hours of acute obstruction of the common bile duct in the cholecystectomised animal most of the bilirubin which accumulates in the liver lymph is of the direct-reacting type. Recent work, referred to above, has shown that direct-reacting bilirubin is composed of two moieties, designated pigments I and II. It has been shown by Hoffman et al. that pigment II predominates in formed canine bile and that it constitutes about 75 per cent. of the direct-reacting bilirubin present therein.

It remained to be seen which type of pigment accumulated in the lymph coming from the liver during the first twenty-four hours of biliary obstruction. Accordingly samples of liver lymph were obtained from three cholecystectomised animals (Nos. 2, 7, 11) during this phase when bilirubin was passing freely into it. These were submitted to analysis by 'reverse phase' chromatography.

Results /

Results

The relative percentages of these direct pigments present specimens of lymph from three animals are given in the following table.

Direct Bilirubin Concentration

Dog No.	Direct Bilirubin Conc. mg.%.	Per Cent. Pigment I.	Per cent. Pigment II
2	11.25	22.0	78.0
7	9.57	28.0	72.0
11	16.66	32.0	68.0
		27.3	72.7

It appeared therefore that about 70 per cent. of the direct pigment present in the lymph under these conditions was of the mature variety designated pigment II which has been shown to predominate in formed canine bile.

SECRETION OF B.S.P. AND BILE INTO THE BILIARY TREE DURING OBSTRUCTION

There was evidence from the above studies on cholecystectomised animals that during the early stages of biliary obstruction the liver was still able to extract B.S.P. and bilirubin from the blood passing through it and to concentrate both. It seemed that after twenty-four hours this mechanism failed. Chromatographic studies had shown that the bilirubin accumulating in the lymph in these early stages was of the mature type predominating in formed bile. It remained to be seen if during this phase, when concentrated bilirubin and B.S.P. were appearing in the liver lymph, secretion of these substances into the biliary tree was actually taking place.

Accordingly B.S.P. was given to six cholecystectomised animals (Nos. 29, 30, 42, 46, 47, 48) with lymphatic fistulae by means of a constant speed injection apparatus. Biliary fistulae also had been established in these preparations by inserting a Transflex tube into the common bile duct and bringing it out through a stab wound in the loin. The tube was obstructed with a metal nail.

In /

In one group (Nos. 29, 30, 42) the injection was given during the early stages of the obstruction when concentrated bilirubin was passing freely into the lymph. It was continued for about three hours and at the end of that time the common bile duct tube was released. The pent-up bile was now collected in a series of graduated centrifuge tubes as it flowed from the catheter. 0.5 ml. of bile was allowed to drain into each receiving vessel so that each 0.5 ml. was collected separately and serially in numbered tubes.

This procedure was followed in a second group of studies on similar preparations (Nos. 46 to 48). Here however at least twenty-four hours were allowed to elapse from the time of obstruction of the common bile duct before the B.S.P. injection was given. Once more at the end of the injection the metal nail was removed and the pent-up bile collected separately in 0.5 ml. quantities. The concentration of B.S.P. in the bile from each of these tubes was now measured.

In a third group of preparations (Nos. 49 to 52) similar to the above a bilirubin study was made. After twenty-four hours of obstruction the common bile duct tube was released and the bilirubin content in the 0.5 ml. serial collections of the bile was determined.

Results /

Results

The capacity of the Transflex tubes inserted into the common bile duct in these preparations was 0.2 ml. So that of the first 0.5 ml. of bile to flow from a tube after release only 0.3 ml. could have come from the biliary tree proper. Part at least of this bile must have represented that from the extra hepatic ducts since the tube had been tied into the distal part of the common bile duct. Nevertheless in the first group of animals (Nos. 29, 30, 42) where B.S.P. had been given two to four hours after obstruction was produced the first 0.5 ml. of bile, which drained off after the tube was released, was found to contain B.S.P. In two of these preparations (Nos. 29, 30) the concentration of B.S.P. in this first 0.5 ml. sample was far in excess of that found in simultaneous samples of lymph or venous blood. In the third animal (No. 42) the second 0.5 ml. draining off contained a similarly high B.S.P. level (Fig.12).

In the second group of animals (Nos. 46 to 48) however in which the B.S.P. injection was withheld until the biliary tree had been obstructed for at least twenty-four hours more than 5.0 ml. of bile had drained off from each catheter before concentrations of B.S.P. equalling those in the lymph or blood /

blood were encountered.

The values plotted along the ordinate in Fig.12 represent the means of the B.S.P. concentrations found in the bile of the three animals comprising a group. The abscissa denotes to which 0.5 ml. specimen these mean figures refer. This graph (Fig.12) gives a clear indication of the differences in distribution of B.S.P. within the biliary tree between these two types of preparation. In the early group where obstruction had been present for about seven hours before it was relieved it can be seen that B.S.P. appeared in the first 0.5 ml. of bile and increased rapidly in concentration thereafter, so that in the third specimen, 1.5 ml. of bile having drained from the fistula, the mean of the concentrations of B.S.P. in these three animals was found to be over 200 mg. per cent. This greatly exceeded the corresponding lymph concentrations which are shown individually as X's. In the second group however where obstruction had been present for about thirty hours it can be seen that even after 5.0 ml. of bile had been drained off the mean concentration of B.S.P. in the last sample in this group was still insignificant and, in fact, lower than the corresponding lymph levels.

In this second group of animals (Nos. 46 to 48) the first one or two tubes contained dark green bile which had been seen stagnating in the Transflex catheter and had presumably also lain /

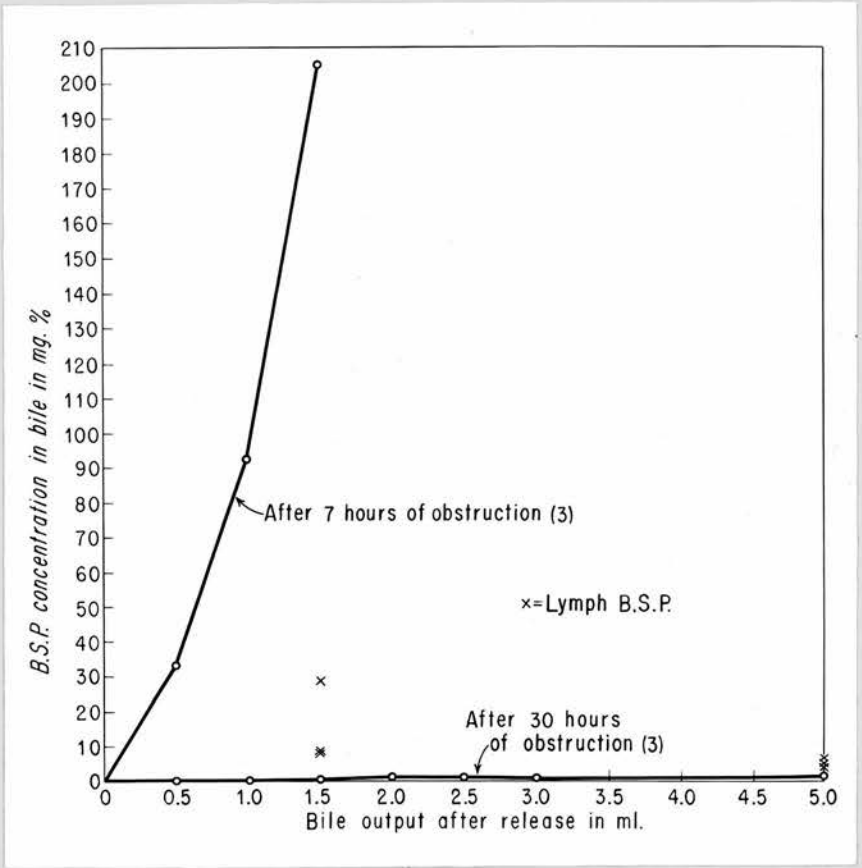


Fig. 12. **Secretion of B.S.P. into the Biliary Tree During Obstruction**

This figure depicts the findings in a series of experiments described under the heading, 'Secretion of B.S.P. and Bile into the Biliary Tree During Obstruction'.

lain in the larger bile ducts. The next eight tubes however contained bile which was more or less colourless. Indeed, most of the specimens had the classical appearance of 'white bile' (Fig.13). The familiar purple colour which B.S.P. imparts to the bile was not seen in these first ten tubes although it appeared soon thereafter. It became clear therefore that little or no bilirubin could be present in this colourless bile.

This was investigated in a further group of cholecystectomised animals (Nos. 49 to 52) to which no B.S.P. had been given. After twenty-four hours or more the common bile duct was released and the same technique was used for collection of the bile. Biliverdin was present in the bile of one of the four preparations (No. 50) used. It has been claimed that the development of such a 'green system' is due to some leak from the biliary tree (McMaster, et al., 1923b). It is possible that in this animal a separate duct emptying into the gall bladder was torn during cholecystectomy and constituted such a leak. On the other hand a leak may have occurred at the site of fixation of the tube into the common bile duct. Post-mortem examination did not disclose evidence that either of these had happened.

Nevertheless bilirubin measurements on the bile collected after /

Fig. 13. Bile from the Ducts of a Cholecystectomised Animal After Thirty Hours of Obstruction

This animal had been given an injection of B.S.P. for three hours (Experiment 48). The common bile duct tube was released and the bile collected in 0.5 ml. quantities as it drained from the tube. The first two tubes (reading from left to right) contained dark green biliverdin which had been stagnating in the tube and in the extrahepatic ducts. The first nine tubes contained no B.S.P. The next tube contained a trace of it and in the last two tubes the purple colour of the drug began to appear.

after release from all four preparations confirmed the B.S.P. findings. A scrutiny of the protocols (Nos. 49 to 52) will show that apart from the first one or two tubes containing dark green bile, which no doubt had been stagnating in the catheter and in the larger biliary ducts, the next 4.0 ml. or so of bile contained either no bilirubin or subnormal amounts of it and not more than was found in the corresponding venous blood samples.

DISCUSSION

The results will be discussed in the order in which they have been presented.

Data obtained during the first twelve hours after acute obstruction of the common bile duct in the recently cholecystectomised animal conform with the findings on thoracic duct fistula animals which have been reported in the literature (Barron and Bumstead, 1928; Bloom, 1923; Caldini and Montagnani, 1953; Gonzalez-Oddone, 1946; Harley, 1893; Heidenhain, 1868; Herring and Simpson, 1907). As might have been expected the changes in the liver lymph were more pronounced. In these preparations it was an invariable finding that during this period a throwback of bilirubin and B.S.P. into the liver lymph took place. It was also clear that a slow but steady increase in the concentration of bilirubin in the blood serum occurred. Part only of this can have been due to leakage of lymph through the hepatic venous lymph system (Appendix A) into the thoracic duct. Since, during the first five hours of biliary obstruction in cholecystectomised dogs having both thoracic duct and liver lymph fistulae (Appendix B), the blood serum concentration of bilirubin more than doubled itself. Conversely it might be argued that the /

the increase could be due to simple retention of indirect bilirubin. In all of these studies however there was a marked preponderance of the direct-reacting pigment in the blood during the first twenty-four hours of obstruction. It seems likely therefore that a throwback of bilirubin into the blood also occurred during this time.

By twenty-four hours however this phase had passed and thenceforth no significant disparity between lymph and blood levels was observed (Gonzalez-Oddone, 1946). Consequently it seemed that, as had been suspected by many previous workers (Barron and Bumstead, 1928; Mayo and Greene, 1929; Reiners, Jr., 1951; Rich, 1930), the period during which these substances accumulated in the lymph was of limited extent.

Previously cholecystectomised animals gave much the same response although, in general, the changes observed in the lymph appeared to be less marked while those in the blood were, if anything, more pronounced.

Studies on animals with the gall bladder <u>in situ</u> however showed no evidence of such accumulations in the lymph. Bollman <u>et al.</u> (1927) have shown that from thirty-six to fifty-six hours after obstruction little change occurs in the concentration of bilirubin in the blood of such preparations and it seems, from the above experiments, that the liver lymph levels follow /

follow suit. In one experiment (Dog No. 22) however lymph levels did rise for a period during the second day. It is possible only to speculate about the explanation for this. Shafiroff et al. (1939) has shown that the level of bilirubin found in the lymph, under these circumstances, increases with the intrabiliary pressure. It is possible that for some reason the pressure rose temporarily and a period of 'abortive regurgitation' ensued.

Bollman et al. (1927) write of failure of reabsorption from the gall bladder as being the most likely cause for the icterus which usually begins to develop about the second or third day. If, however, this was the only factor involved then the resulting situation would be much the same as that which obtains during acute obstruction of the common bile duct in the cholecystectomised animal and a throwback into the lymph would then be anticipated. This did not occur. It may be that the failure is incomplete and that sufficient reabsorption still goes on to prevent the development of a rise in intrabiliary pressure large enough to cause a throwback. It is equally possible however that secretion into the biliary tree is reduced or, indeed, that it ceases altogether. This will be discussed below.

Studies of the effect of a second period of obstruction in cholecystectomised animals showed that a previous bout lasting /

lasting for three hours was sufficient to prevent the accumulation of bilirubin and B.S.P. in the lymph when the obstruction was renewed twenty-four hours later. It may be that, if it had been possible to keep these preparations functioning satisfactorily for several days longer before renewing the obstruction the expected accumulation of these substances in the lymph would have occurred. At the moment it does not seem to be technically possible to keep these animals going for this length of time. Nevertheless it appears that some fundamental change follows this first three hour period of obstruction. A second period of obstruction on the next day produced an effect which in no way differed from that which would have been found, at this stage, had the obstruction never been relieved. This suggests that the first short episode of obstruction has effects which seem to be more or less irreversible during this time since relief of the obstruction does not modify them.

Chronic obstruction of the common bile duct, whether the gall bladder was present or not, gave liver lymph levels of bilirubin and P.S.P. which were lower than those in the blood. This confirms the original observation made by Whipple and King, in 1911. Using the Salkowski test they found that the pericardial fluid and the thoracic duct lymph from dogs with chronic /

chronic obstruction contained less bilirubin than the blood serum.

The rat experiments confirmed the above findings with the exception that no great preponderance in the concentration of bilirubin in the liver lymph occurred during the first twenty-four hours. It may be that having no gall bladder the biliary tree in these animals has a greater intrinsic absorptive potential. To date no observations on this subject have appeared in the literature. At all events it is perhaps not incongruent that the response to biliary obstruction in the rat was similar to that seen in the dog with its gall bladder in situ.

It appeared therefore from these studies that a definite pattern could in fact be discerned. Theoretically, when the concentration of bilirubin or B.S.P. in the liver lymph significantly exceeds that of the venous blood regurgitation from the biliary tree into the lymph is a possibility. It is clear from the above studies that this could only take place during the first twenty-four hours of biliary obstruction in animals from which the gall bladder had been removed. Up to five weeks after this time there was no significant difference between blood and lymph levels of these substances and this was also the case in acute and chronic obstruction when /

when the gall bladder was present.

It seems therefore that such is the Standard Response to Biliary Obstruction in terms of the measurements made in the above studies.

From the data obtained in the studies discussed above no conclusion can be drawn as to whether or not, after the first twenty-four hours, regurgitation into the blood takes place. It became necessary therefore to find cut if the liver was still able to concentrate bilirubin and B.S.P. when this first period of twenty-four hours had passed.

The injection of B.S.P. during the phase when bilirubin was passing in high concentrations into the liver lymph showed that it was extracted from the blood passing through the liver concentrated and somehow diverted into the lymph. This was accompanied by a fall in the concentration of bilirubin therein and stopping the injection produced a secondary rise in the level of bilirubin in the lymph. It seemed therefore that the liver was still able to concentrate bilirubin and B.S.P. at this stage. Further, since the liver had extracted B.S.P. from the blood passing through it it was reasonable to assume that during this phase it was also extracting bilirubin. The demonstration that the introduction of B.S.P. into /

into this system had impaired its ability to concentrate bilirubin was similar to that made by Cantarow et al. (1948). These workers determined the rate of bile flow and pigment excretion in Thomas type duodenal fistula dogs and showed that at elevated serum bilirubin concentrations bilirubin and B.S.P. compete for a common excretory mechanism.

Chromatographic methods showed that the direct pigment accumulating in the liver lymph during the first twenty-four hours of obstruction in the cholecystectomised animal was mainly of the mature type, designated pigment II by Billing (1955a). It has been demonstrated that this variety predominates in fresh human and canine bile and, consequently, since the bilirubin present in the lymph was mainly in the direct form this seemed to be good, but not conclusive evidence that it had come from the biliary tree.

It was now observed that B.S.P. given during the first twenty-four hours of obstruction in the cholecystectomised animal appeared in high concentrations in the first millilitre of bile which drained from the common bile duct tube when it was released. Thus it became clear that during this phase B.S.P. was still being excreted in concentrated form into the bile.

When however it was given after the first twenty-four hours /

hours of obstruction more than 5.0 ml. of bile had flowed from the tube before concentrations of B.S.P. equalling those in the liver lymph or the blood were encountered. This is in accord with the findings of Snell et al. (1925) who gave repeated doses of B.S.P. to dogs with chronic biliary obstruction but could find no dye in the bile passages of these animals afterwards.

It was noticed during the previous study that the first 5 ml. of bile which drained from the common bile duct tube after release was colourless (Kausch, 1911; McMaster et al. 1923b) and hence it seemed likely that it contained little or no bilirubin (Fig. 13). A similar study was now carried out and here the bilirubin content of this bile was analysed. Apart from the first millilitre or so, which contained both bilirubin and biliverdin, which had been seen stagnating in the tube and presumably also had lain in the distal part of the extrahepatic biliary tree, the first 5 ml. of this bile was found to contain either no bilirubin or no more than was present in the blood at that time.

Brauer and Pessotti (1950) have pointed out that figures for intrahepatic bile volumes do not exist in the literature. According to Bollman (Personal communication) and Maegraith (Personal communication) the capacity of the whole biliary tree in /

im a 10 kg. dog, is about 5 ml. If these observations are correct then no bilirubin or B.S.P. could have been present in the biliary tree of these animals. The possibility remains however that this did not represent all of the bile from the biliary tree. It could be that, at the canalicular level, normal secretion was still going on. But it was clear from the above studies, that even if this was happening regurgitation of normal bile from the canaliculi into the liver lymph was not taking place, since the concentrations of both bilirubin and B.S.P. in the lymph at this stage were not found to be significantly higher than the blood levels of these substances. It seemed therefore that the liver, by this time, had ceased to concentrate bilirubin. Consequently the point is no more than an academic one since, even if regurgitation from the biliary tree into the lymph was still going on, the concentration of the substances being regurgitated could be no higher than those present in the blood. Hence such regurgitation could not increase the degree of icterus already present.

It might be argued that now regurgitation into the blood alone was taking place and that by twenty-four hours the pathways for regurgitation into the lymph had become blocked. It would be extremely difficult to sustain this argument on a histological basis, since so little is known about the pathways /

pathways themselves. Nevertheless an attempt must be made to answer the criticism. It has been shown that a second period of obstruction at this stage is not followed by the passage of bilirubin or B.S.P., in concentrated form, into the lymph. This occurs if the biliary tree has been obstructed for only three hours on the previous day. Hence, if the above criticism is valid, it would follow that the pathways for regurgitation into the lymph must already have become blocked by the end of the first three hour period of obstruction. This is certainly not the case since it has been repeatedly demonstrated in the above studies that if obstruction is maintained for more than three hours the passage of bilirubin and B.S.P. into the lymph continues.

It appears therefore that after twenty-four hours of obstruction the liver can no longer concentrate bilirubin or B.S.P. Moreover it seems that secretion into the biliary tree becomes suspended after this time. Nevertheless the mature pigment II accumulates in the blood (Billing, 1955b; Hoffman et al, 1957). Hence it seems likely that the liver cell is still able to effect the conversion of pigment I into pigment II (Hoffman et al, 1957).

If these observations and the conclusions derived from them are correct it follows that, after the regurgitation phase /

phase which takes place during the first twenty-four hours of obstruction in the cholecystectomised animal, bilirubin still continues to enter the hepatic cell and is changed by it into the mature pigment II. It seems likely that it is then returned directly to the blood without being excreted into the biliary tree. Indeed, it has been claimed that this is part of the normal mechanism of biliary excretion (Andrews, 1955). This, of course, is no more than an amplification of the theory put forward in 1892 by Minkowski (1892, 1895, 1904) and termed by him 'paracholie'. It was his belief that during biliary obstruction a reversal of polarity occurred in secretion from the hepatic cell and that the bile was now passed directly into the sinusoids. The evidence obtained suggests that this may be the Mechanism of the Standard Response.

SUMMARY

(1) This investigation was of the nature of an experimental study on the pathogenesis of surgical (obstructive) jaundice. Unanaesthetised canine and rat preparations, having liver lymphatic fistulae, were used.

(2) An attempt was made to reproduce experimentally the usual circumstances under which biliary obstruction occurs in man. Hence three types of canine preparations were used:-

(a) Recently cholecystectomised animal.

(b) Previously cholecystectomised animal.

(c) Animal with gall bladder in situ.

and the following varieties of obstruction were studied:-

(a) Acute obstruction (canine and rat experiments)

(b) Recurrent acute obstruction (canine experiments)

(c) Chronic obstruction (canine and rat experiments)

(3) Measurements were made of the resulting effect on the concentrations of bilirubin in the lymph coming from the liver and in the systemic venous blood.

(4) Canine studies showed that:-

(a) In the recently and previously cholecystectomised animal a phase of regurgitation of bilirubin into the liver lymph followed acute obstruction of the common bile duct. This phase lasted for about twenty-four hours. Thereafter /

Thereafter and throughout chronic obstruction there was no significant difference between the concentration of bilirubin in either the lymph or the blood. Moreover, a first period of obstruction lasting for three hours abolished this phase when the obstruction was renewed next day.

(b) In the animal with its gall bladder in situ no such phase of regurgitation was observed during either acute or chronic biliary obstruction. Little change took place in the concentration of bilirubin in either lymph or blood during the first two or three days and then a gradual rise occurred, both levels being approximately equal throughout.

(c) Studies during which bromsulphthalein (B.S.P.) was given intravenously by a constant speed injection apparatus, to animals with acute, recurrent acute and chronic obstruction of the common bile duct, gave similar results.

(5) In the rat experiments this phase of regurgitation of bilirubin into the liver lymph was not apparent. After the first twenty-four hours and during chronic obstruction the /

the findings were similar to those of the canine experiments.

(6) Canine studies on the mechanism of the responses summarised above showed that:-

(a) During the first twenty-four hours of obstruction in the cholecystectomised animal, the liver was still able to extract B.S.P. from the circulating blood and to concentrate both bilirubin and B.S.P.

(b) After twenty-four hours it appeared to be unable to do this.

(c) When B.S.P. was introduced into this system during the early hours of biliary obstruction it competed successfully against bilirubin for the concentrating mechanism.

(d) Pigment II (Billing, 1955b) predominated in the liver lymph during the regurgitation phase.

(e) When B.S.P. was given to cholecystectomised animals during the regurgitation phase it was found in high concentrations in the first millilitre of bile draining from the common bile duct tube upon relief of the obstruction.

(f) /

(f) When, however, B.S.P. was given after obstruction had been present for more than twenty-four hours, the first 5.0 ml. of bile draining from the common bile duct tube was found to be colourless and to contain little or no B.S.P. A similar study, where no injection of B.S.P. was given showed that the first 5.0 ml. of bile which drained off contained insignificant amounts of bilirubin.

CONCLUSIONS

These findings do not support the view that throughout biliary obstruction bile continues to be secreted into the biliary tree and to be 'regurgitated' from there into the blood and lymph (Rich, 1930), or into the lymph only (Watson, 1940, 1955).

Regurgitation comes to an end when biliary obstruction has been present for about twenty-four hours in the cholecystectomised dog. Thereafter and throughout chronic obstruction it does not occur. Nor is it seen during acute or chronic biliary obstruction in the dog which has its gall bladder *in situ* or in the rat.

It appears that at an early stage during the development of surgical jaundice the canine liver becomes unable to concentrate bilirubin and secretion of bile into the biliary tree is discontinued.

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PROTOCOLS

CANINE EXPERIMENTS 1 to 52.

RAT EXPERIMENTS A to L.

BILIRUBIN (Acute G.B.* out)

Experiment 1 Op. finished 10 a.m. G.B. out
Dog 1 C.B.D.** Tube
16.4.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.84	0.11 (15' direct)
		0.95	0.12 (total)
	12 noon	0.94	0.11
		1.22	0.12
	2 p.m. Obstruct C.B.D. Tube		
	2.30 p.m.	0.95	0.21
		2.50	0.25
	3 p.m.	4.37	0.21
		6.12	0.30
	4 p.m.	5.36	0.30
		7.75	0.37
	5 p.m.	17.82	0.54
		21.50	0.70
	6 p.m.	23.43	0.72
		27.00	1.20
	7 p.m.	17.16	1.05
		18.75	1.35
	10 p.m.	8.91	1.78
		9.00	2.50
17.4.56	10 a.m.	3.38	2.09
		3.75	2.50
	1.30 p.m.	3.30	2.21
		3.82	2.37
	2 p.m. Ether anaesthesia induced		
	2.30 p.m.	3.43	2.04
		3.75	2.45
	3 p.m.	2.54	1.80
		3.25	2.15

3 /

(* G.B. = gall bladder; ** C.B.D. = common bile duct)

Experiment 1 (Contd.)
Dog 1

	Time	Lymph mg. %	Blood mg. %
17.4.56	3 p.m. Stop anaesthetic		
	3.30 p.m.	2.31	2.17
	(Conscious)	2.95	2.35
	4 p.m.	2.64	2.50
		3.25	3.05
	4.30 p.m.	2.47	2.50
		2.85	2.70
	5 p.m.	2.60	2.54
		3.25	2.75
18.4.56	10 a.m.	3.21	3.46
		4.12	4.25
	5 p.m.	3.71	4.45
		4.50	5.12
19.4.56	10 a.m.	4.53	5.69
		5.62	6.50

BILIRUBIN (Acute G.B. out)

Experiment 2 Op. finished 10 a.m. G.B. out
Dog 2 C.B.D. Tube
12.4.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.31	0.06
		0.37	0.06
	12 noon	0.66	0.13
		0.75	0.17
	1 p.m. Obstruct C.B.D. Tube		
	2.30 p.m.	5.94	0.23
		8.00	0.40
	3 p.m.	9.40	0.28
		10.75	0.28
	3.30 pm.	10.23	0.21
		11.25	0.22
	4 p.m.	11.25*	0.37
		13.50	0.75
13.4.56	10 a.m.	3.05	2.64
		3.62	3.00
	3 p.m.	3.05	2.64
		3.50	3.00
14.4.56	10 a.m.	2.80	2.64
		3.00	2.80
	2 p.m.	2.77	2.83
		3.00	3.75
15.4.56	10 a.m.	2.95	3.75
		3.50	4.05

*Chromatographic analysis of the specimen of lymph obtained at 4 p.m. on 12.4.56 showed it to contain 22% pigment I and 78% pigment II.

BILIRUBIN (G.B. out)

Experiment 3 Op. finished 10 a.m. G.B. out
Dog 3 Lig. & Div. C.B.D.
16.7.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	3 p.m.	9.3	-
		11.0	-
	4 p.m.	11.99	-
		12.7	-
	7 p.m.	12.37	-
		13.4	-
	12 midnight	11.22	-
		11.75	-
17.7.56	8 a.m.	8.58	-
		8.8	-
	10 a.m.	6.76	4.62
		7.5	5.0
	12 noon	6.79	-
		7.1	-
	3 p.m.	5.28	5.28
		6.2	6.7
	6 p.m.	4.62	-
		6.0	-
	8 p.m.	4.95	5.08
		5.4	5.65
18.7.56	10 a.m.	4.07	4.07
		4.6	4.6
	2 p.m.	3.87	-
		3.9	-
	6 p.m.	3.13	3.79
		3.8	4.12
19.7.56	8 a.m.	3.79	3.97
		4.20	4.20
	10 a.m.	2.74	4.1
		3.5	4.75

Experiment 3 (contd.)
Dog 3

	Time	Lymph mg. %	Blood mg. %
19.7.56	12 noon	3.2	4.15
		4.05	4.70
	2 p.m.	4.62	-
		5.62	-
	4 p.m.	4.95	4.95
		5.62	5.75
	8 p.m.	4.20	6.18
		5.37	7.25

BILIRUBIN (Acute G.B. out)

Experiment 4 Op. finished 10 a.m. G.B. out
Dog 4 C.B.D. Snare
25.4.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.37	0.04
		0.51	0.07
	12 noon	0.21	0.04
		0.32	0.07
	5 p.m.	0.33	0.04
		0.50	0.07
26.4.56	10 a.m.	0.13	0.04
		0.30	0.07
	11 a.m.	Obstruct C.B.D. with Snare	
	12 noon	2.90	0.09
		4.15	0.15
	1 p.m.	18.81	0.21
		21.00	0.27
	2 p.m.	17.82	0.24
		19.50	0.30
	3 p.m.	18.48	0.33
		21.75	0.42
	4 p.m.	23.10	0.49
		24.25	0.62
	5 p.m.	19.14	0.69
		20.75	0.85
	6 p.m.	18.31	0.74
		19.75	0.95
	8 p.m.	16.50	1.02
		18.25	1.27
27.4.56	10 a.m.	2.97	2.14
		3.50	2.50
	3 p.m.	1.99	1.98
		2.50	2.50
28.4.56	10 a.m.	Lymph catheter blocked	2.14
			2.49

BILIRUBIN (Acute G.B. out)

Experiment 5 Op. finished 10 a.m. G.B. out
Dog 5 C.B.D. Tube
18.4.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.37	0.08
		0.37	0.12
	12 noon	0.24	0.08
		0.27	0.12
	5 p.m.	0.56	0.06
		0.60	0.07
19.4.56	10 a.m.	0.67	0.13
		1.40	0.20
20.4.56	C.B.D. tube not draining well		
	10 a.m.	2.12	0.43
		2.55	0.96
	2 p.m. Obstruct C.B.D. tube		
	3.30 p.m.	2.31	0.66
		2.75	1.12
	4.30 p.m.	4.78	1.07
		5.62	1.32
	7 p.m.	8.91	1.89
		11.12	2.50
21.4.56	10 a.m.	4.04	4.37
		4.62	5.00

BILIRUBIN (G.B. out. Vinyl ac.)

Experiment 6 — Op. finished — G.B. out
Dog 6 — 10 a.m. — *6 ml. Vin. ac.C.B.D.
25.6.56 — Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	At op.	0.99	-
		1.25	
	11 a.m.	3.13	0.08
		3.37	0.12
	12 noon	5.74	0.14
		6.5	0.17
	1 p.m.	8.49	0.19
		9.12	0.3
	2 p.m.	10.39	-
		11.5	-
	3 p.m.	10.5	-
		11.75	-
	4 p.m.	11.30	-
		12.4	-
	5 p.m.	11.8	0.39
		13.0	0.5
	7 p.m.	14.68	-
		16.0	-
	8 p.m.	14.8	0.49
		16.25	0.75
	10 p.m.	16.5	0.99
		17.5	1.25
26.6.56	10 a.m.	6.76	2.47
		7.25	3.0

(* 6 ml. of Vinyl acetate were rejected into the common bile duct at operation.)

BILIRUBIN (Prev. Cholecystectomised)

Experiment 7 Op. finished 10 a.m. G.B. out
Dog 7 C.B.D. tube
6.6.56 Liver lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.47	0.08
		0.57	0.09
	2 p.m.	Obstruct C.B.D. tube.	
	4 p.m.	9.57*	0.34
		11.62	0.45
	6 p.m.	10.23	0.75
		11.75	1.00
	8 p.m.	8.01	1.089
		11.15	1.30
	10 p.m.	10.23	1.11
		11.5	1.35
	12 midnight	9.65	2.36
		10.85	2.62
7.6.56	4 a.m.	4.85	-
		5.75	
	8 a.m.	3.87	4.04
		4.64	4.57
	12 noon	4.37	4.70
		5.62	5.5
	2 p.m.	4.70	6.02
		5.87	6.75

*Chromatographic analysis of the specimen of lymph obtained at 4 p.m. on 6.6.56 showed it to contain 28% pigment I and 72% pigment II.

BILIRUBIN (Prev. Cholecystectomised)

Experiment 8	Op. finished 10 a.m.	G.B. out 21.6.56
Dog 8	Lig. & Div C.B.D.	Lig. & Div. C.B.D.
10.7.56	9.45 a.m.	Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	12 noon	2.45	0.12
		2.87	0.27
	2 p.m.	4.45	0.57
		4.87	0.6
	4 p.m.	3.3	0.52
		3.6	0.62
	7 p.m.	4.45	0.74
		5.12	0.87
	9 p.m.	4.25	0.89
		4.98	1.02
	12 midnight	3.84	0.96
		4.02	1.12
11.7.56	10 a.m.	2.72	3.3
		3.35	3.95
	2 p.m.	2.84	3.54
		3.65	4.0
	10 p.m.	3.35	3.38
		4.1	4.5
12.7.56	10 a.m.	4.84	5.2
		5.05	5.75

BILIRUBIN (Prev. Cholecystectomised)

Experiment 9 Op. finished 10 a.m. G.B. out
Dog 9 Snare C.B.D.
8.6.56 Liver Lymph

Time	Lymph mg. %	Blood mg. %
11 a.m.	1.27	0.07
	1.42	0.11
2 p.m.	Obstruct C.B.D. with snare	
3 p.m.	5.87	-
	7.8	-
4 p.m.	5.68	0.478
	7.5	0.57
5 p.m.	6.43	-
	7.12	-
6 p.m.	7.26	0.74
	10.85	0.87
8 p.m.	12.7	0.9
	14.0	1.12
9.30 p.m.	15.8	0.9
	16.0	1.12
12 midnight	14.5	1.4
	15.5	2.0
2 a.m.	12.2	-
	14.0	-
9.6.56 10 a.m.	Lymph catheter blocked	5.11
		5.5

BILIRUBIN (Prev. Cholecystectomised)

Experiment 10	Op. finished 10 a.m.	G.B. out 21.6.56
Dog 10	Lig. & Div. C.B.D.	Lig. & Div. C.B.D.
9.7.56	9.45 a.m.	Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	12 noon	2.02	0.075
		2.56	0.125
	2 p.m.	5.6	0.41
		6.0	0.45
	4 p.m.	6.3	0.58
		6.8	0.63
	6 p.m.	6.1	0.64
		6.75	0.71
	8 p.m.	6.4	0.9
		7.05	1.02
	10 p.m.	5.2	-
		5.96	-
	12 midnight	5.25	1.45
		5.84	1.68
10.7.56	10 a.m.	4.95	5.125
		5.05	5.4
	12 noon	5.12	5.25
		5.2	5.4
	4 p.m.	4.95	5.3
		5.25	5.49

BILIRUBIN (Prev. Cholecystectomised)

Experiment 11	Op. finished	G.B. out
Dog 11	10.30 a.m.	Lig. & Div. C.B.D.
11.6.56	Lig. C.B.D. 10.15am.	Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11.30 a.m.	0.39	0.006
		0.55	0.025
	12.30 p.m.	0.41	-
		0.67	-
	2 p.m.	3.1	-
		4.2	-
	3 p.m.	6.43	0.18
		7.5	0.27
	4 p.m.	9.98	-
		11.62	-
	5 p.m.	14.25	-
		15.5	-
	6 p.m.	11.6	0.19
		13.75	0.35
	8 p.m.	16.66*	0.49
		18.75	0.62
	10 p.m.	15.34	-
		18.25	-
	12 midnight	13.36	0.99
		14.5	1.15
12.6.56	4 a.m.	6.76	-
		7.5	-
	8.30 a.m.	4.29	4.15
		4.8	4.5
	10.15 a.m.	4.62	4.68
		4.8	5.5
	2 p.m.	4.6	5.3
		5.5	6.2
	6 p.m.	4.48	4.81
		4.75	5.25
	12 midnight	4.48	4.64
		5.25	6.25

*Chromatographic analysis of the specimen of lymph obtained at 8 p.m. on 11.6.56 showed it to contain 32% pigment I and 68% pigment II.

BILIRUBIN (Acute. Prev. Cholecystectomised)

Experiment 12 Op. finished 11 a.m. G.B. out 6.4.56
Dog 12 (Prev. Cholecyst.) Snare C.B.D. 30.4.56
30.4.56 Liver Lymph 30.4.56

	Time	Lymph mg. %	Blood mg. %
	12 noon	0.67	0.07
		1.05	0.10
	1 p.m.	0.47	0.07
		0.80	0.09
	5 p.m.	0.44	0.08
		0.72	0.12
1.5.56	10 a.m.	0.36	0.08
		0.45	0.07
	11 a.n. Obstruct C.B.D. with Snare		
	1 p.m.	2.72	0.28
		3.75	0.47
	2 p.m.	4.95	0.82
		5.25	0.92
	3 p.m.	4.95	0.99
		6.25	1.25
	5 p.m. Lymph catheter blocked		1.37
			1.90
	12 midnight	-	3.13
		-	3.60
2.5.56	11 a.m.	-	4.04
		-	4.12
	5 p.m.	-	4.04
		-	4.50
3.5.56	10 a.m.	-	4.04
		-	4.62
	5 p.m.	-	4.45
		-	5.00
4.5.56	10 a.m.	-	4.12
		-	5.50

BILIRUBIN (G.B. in)

Experiment 13 Op. finished 10 a.m. G.B. in
Dog 13 Lig. & Div. C.B.D.
13.6.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.41	0.049
		0.68	0.07
	2 p.m.	0.14	0.04
		0.17	0.7
	7.30 p.m.	0.26	0.09
		0.26	0.14
	12 midnight	0.66	0.12
		0.74	0.35
14.6.56	8 a.m.	0.44	0.29
		0.62	0.35
	10 a.m.	0.44	0.29
		0.62	0.35
	2 p.m.	0.36	0.26
		0.70	0.45
	6 p.m.	0.59	0.36
		0.85	0.5
	12 midnight	0.75	0.42
		1.1	0.76
15.6.56	5 a.m.	1.54	-
		2.1	-
	10 a.m.	2.14	2.44
		2.8	2.95
	12 noon	2.54	2.9
		3.24	3.64
	2 p.m.	2.30	3.1
		3.1	3.96
	4 p.m.	2.8	3.5
		3.5	4.1
	7 p.m.	3.32	3.99
		4.03	4.66
	12 midnight	3.59	4.15
		4.45	5.0
16.6.56	10 a.m.	3.38	5.03
		4.2	5.9

BILIRUBIN (G.B. in)

Experiment 14 Op. finished 11 a.m. G.B. in
Dog 14 Lig. & Div. C.B.D.
28.5.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	12 noon	0.66	0.049
		0.77	0.075
	3 p.m.	0.099	0.099
		0.15	0.125
	6 p.m.	0.099	0.082
		0.16	0.125
	9 p.m.	0.033	0.082
		0.125	0.1
	12 midnight	0.108	0.082
		0.17	0.12
29.5.56	5 a.m.	0.165	-
		0.27	-
	10 a.m.	0.214	0.082
		0.325	0.125
	12 noon	0.165	Haemolysis
		0.35	
	3 p.m.	0.176	0.099
		0.275	0.125
	6 p.m.	0.297	0.016
		0.375	0.125
		Catheter blocked	
30.5.56	10 a.m.	-	0.105
		-	0.225
	12 noon	-	0.165
		-	0.225
	6 p.m.	-	0.165
		-	0.2
31.5.56	12 noon	-	0.264
		-	0.325

BILIRUBIN (G.B. in)

Experiment 15 Op. finished 10 a.m. G.B. in
Dog 15 Lig. & Div. C.B.D.
25.5.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
At op.	9.30 a.m.	0.089	0.016
		0.125	0.025
	10 a.m.	Ligate and Divide Common Bile Duct	
	12 noon	0.176	0.082
		0.2	0.1
	3 p.m.	0.171	0.072
		0.25	0.1
	6 p.m.	0.165	0.082
		0.165	0.125
	9 p.m.	0.285	0.148
		0.325	0.15
	12 midnight	0.33	-
		0.4	-
26.5.56	10 a.m.	0.33	0.25
		0.42	0.27

Lymph catheter blocked

BILIRUBIN (G.B. in)

Experiment 16 Op. finished 10 a.m. G.B. in
Dog 16 Snare C.B.D.
23.5.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	1.08	Haemolysis
		1.5	
	12 noon	0.59	-
		0.7	
	3 p.m.	0.59	0.11
		0.7	0.13
	3 p.m.	Obstruct C.B.D. with snare	
	4 p.m.	0.66	-
		0.9	-
	5 p.m.	0.78	-
		1.04	-
	6 p.m.	0.825	0.148
		1.15	0.17
	7 p.m.	0.396	-
		1.150	
	8 p.m.	0.79	-
		1.2	-
	12 midnight	0.46	0.16
		1.05	0.25
24.5.56	4 a.m.	0.36	-
		0.95	-
	8 a.m.	0.59	0.19
		1.15	0.32
	11 a.m.	0.72	0.33
		1.3	0.45
	3 p.m.	0.89	0.263
		1.25	0.50
	6 p.m.	0.68	0.55
		1.4	0.75
		Dog pulled out lymphatic cannula.	

BILIRUBIN (G.B. in)

Experiment 17 Op. finished 10 a.m. G.B. in
Dog 17 Snare C.B.D.
7.5.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	12 noon	0.8? (reading 0.8 late)	0.066 0.075
	5 p.m.	0.165 0.55	0.099 0.1
	5 p.m.	Obstruct C.B.D. with snare	
	8 p.m.	0.176 0.65	0.016 0.125
	12 midnight	0.265 0.5	0.082 0.15
	2 a.m.	0.33 0.5	- -
8.5.56	10 a.m.	Lymph catheter blocked	0.099 0.15
	5 p.m.	- -	0.165 0.225

BILIRUBIN (G.B. in)

Experiment 18 Op. finished 10 a.m. G.B. in
Dog 18 Snare C.B.D.
20.6.56 Liver Lymph

Time	Lymph mg. %	Blood mg. %
11 a.m.	0.44	0.07
	0.52	0.12
3 p.m.	Obstruct C.B.D. with snare	
5 p.m.	0.37	0.099
	0.425	0.125
10 p.m.	0.28	0.09
	0.32	0.12
21.6.56	Lymph catheter blocked	
3 p.m.	-	0.11
	-	0.15

BILIRUBIN (Acute G.B. in)

Experiment 19 Op. finished 10 a.m. G.B. in
Dog 19 Snare C.B.D.
2.5.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	0.92	0.07
		1.12	1.50
	12 noon	0.85	0.04
		1.20	0.07
	5 p.m.	0.33	0.08
		0.60	0.12
3.5.56	10 a.m.	0.24	0.04
		0.57	0.15
	3 p.m.	0.12	0.06
		0.55	0.12
	3 p.m. Obstruct C.B.D. with snare		
	5 p.m. Lymphatic blocked		0.06
		-	0.15
	8 p.m.	-	0.04
		-	0.07
	12 midnight	-	0.16
		-	0.25
4.5.56	10 a.m.	-	0.75
		-	0.90
	3 p.m.	-	0.85
		-	1.05
5.5.56	10 a.m.	-	1.10
		-	1.40
	3 p.m.	-	1.15
		-	1.44

BILIRUBIN (G.B. in)

Experiment 20 Op. finished 10 a.m. G.B. in
Dog 20 (Lig. & Div. C.B.D. Lig. & Div. C.B.D.
29.6.56 24 hr. prev.) 28.6.56
Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	Pre-op.	-	0.39
		-	0.6
	At op.	2.52	-
		2.60	-
	Post-op.		
	11 a.m.	1.08	-
		1.4	-
	12 noon	0.49	0.29
		0.5	0.3
	2 p.m.	0.56	-
		0.7	-
	4 p.m.	0.69	-
		0.85	-
	6 p.m.	0.62	0.29
		0.8	0.38
	8 p.m.	0.66	-
		0.87	-
	12 midnight	0.99	0.58
		1.1	0.65
30.6.56	4 a.m.	1.02	-
		1.1	-
	8 a.m.	1.07	0.74
		1.1	0.80
	10 a.m.	1.12	0.90
		1.20	0.98
	12 noon	1.36	1.30
		1.54	1.46
	2 p.m.	1.90	1.78
		1.98	1.90

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Experiment 20 (contd.)
Dog 20

	Time	Lymph mg. %	Blood mg. %
30.6.56	4 p.m.	2.04	2.18
		2.26	2.46
	6 p.m.	2.40	2.48
		2.68	2.82
	10 p.m.	3.65	3.98
		3.81	4.09
	12 midnight	3.79	4.07
		3.91	4.15
1.7.56	8 a.m.	5.03	5.33
		5.29	5.58

Lymph catheter blocked

BILIRUBIN (G.B. in)

Experiment 21 Op. finished 10 a.m. G.B. in
Dog 21 (Lig. & Div. C.B.D. Lig. & Div. C.B.D.
5.7.56 48 hr prev.) 3.7.56
Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	Pre-op.	-	2.12
		-	2.25
	At op.	5.6	-
		6.0	-
	Postop.		
	11 a.m.	3.96	1.81
		4.5	2.12
	2 p.m.	1.65	1.23
		2.0	1.5
	4 p.m.	1.02	-
		1.12	-
	6 p.m.	1.07	0.75
		1.3	0.85
	9 p.m.	0.9	0.66
		1.12	0.75
6.7.56	10 a.m.	0.90	0.49
		1.12	0.5
	2 p.m.	0.82	0.59
		1.0	0.75
	10 p.m.	1.10	0.74
		1.32	1.12
7.7.56	10 a.m.	1.40	1.23
		1.62	1.5

BILIRUBIN (G.B. in)

Experiment 22
Dog 22
18.7.56

Op. finished 10 a.m.
(Lig. & Div. C.B.D.
24 hr. prev.)

G.B. in
Lig. & Div. C.B.D.
17.7.56
Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	10 a.m.	2.93	0.52
		3.2	1.0
	12 noon	1.48	-
		1.75	-
	2 p.m.	0.99	-
		1.17	-
	4 p.m.	0.74	0.74
		1.15	1.125
	6 p.m.	0.41	-
		0.76	-
	12 midnight	1.68	0.98
		1.90	1.36
	Overnight sample less than 5.0		
19.7.56	8 a.m.	4.6	1.58
		5.25	2.08
	12 noon	3.54	1.41
		4.27	1.82
	4 p.m.	3.13	2.06
		4.0	2.25
	8 p.m.	2.47	1.74
		2.8	2.12
20.7.56	10 a.m.	4.18	4.53
		4.90	5.25

BILIRUBIN (G.B. in)

Experiment 23
Dog 23
26.7.56

Op. finished 10 a.m.
(Lig. & Div. C.B.D.
24 hr. prev.)

G.B. in
Lig. & Div. C.B.D.
25.7.56
Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	10 a.m.	4.37	0.59
		4.62	0.62
	12 noon	0.99	-
		1.12	-
	2 p.m.	1.51	-
		2.0	-
	4 p.m.	1.51	0.49
		2.12	0.62
	8 p.m.	1.15	0.49
		1.62	0.62
	12 midnight	1.32	0.60
		1.68	0.78
27.5.56	10 a.m.	1.23	0.74
		1.62	0.87
	2 p.m.	1.32	0.79
		1.85	0.85
		Lymph catheter blocked	

BILIRUBIN (G.B. in)

Experiment 24
Dog 24
9.8.56

Op. finished 10 a.m.
(Lig. & Div. C.B.D.
48 hr. prev.)

G.B. in
Lig. & Div.
C.B.D. 7.8.56
Liver Lymph

Time	Lymph mg. %	Blood mg. %
10 a.m.	5.35	-
	5.70	-
11 a.m.	3.05	1.12
	3.15	1.56
12 noon	2.14	-
	2.37	-
1 p.m.	1.98	1.02
	2.12	1.18
4 p.m.	1.56	1.10
	1.92	1.24

Lymph catheter blocked

BILIRUBIN (G.B. in)

Experiment 25
Dog 25
31.8.56

Op finished 10 a.m.
(Lig. & Div. C.B.D. 28.8.56. Three-day obstruction, G.B. in)

G.B. in Lig. & Div. C.B.D. 28.8.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	10 a.m.	3.97	3.37
		3.62	4.37
	11 a.m.	3.12	-
		3.55	-
	2 p.m.	2.97	3.30
		3.62	4.12
	5 p.m.	3.79	3.79
		4.75	4.75
	8 p.m.	3.87	3.87
		4.75	4.75
	12 midnight	3.98	4.36
		4.86	4.96
1.9.56	10 a.m.	4.24	4.48
		4.49	5.16

BILIRUBIN (Acute G.B. out)

Experiment 26 Op. finished 10 a.m. G.B. out.
Dog 26 C.B.D. Tube.
21.3.56 Can. Portal Vein.
Liver Lymph

Time	Lymph mg. %	Blood (Portal Vein) mg. %
10.30 a.m.	0.24	0.03
	0.46	0.03
11.15 a.m.	0.23	0.15
	0.25	0.17
12.5 p.m. Obstruct C.B.D. Tube		
12.40 p.m.	0.18	0.09
	0.25	0.13
1 p.m.	0.21	0.09
	0.32	0.10
1.20 p.m.	0.42	0.05
	0.52	0.07
1.40 p.m.	0.70	0.05
	0.92	0.05
2 p.m.	0.77	0.09
	1.22	0.12
2.20 p.m.	0.84	0.09
	1.25	0.15
2.40 p.m.	0.95	0.19
	1.60	0.25
3 p.m.	1.57	0.11
	2.35	0.22
3.5 p.m. Release C.B.D. Tube		
3.20 p.m.	1.43	0.21
	1.95	0.25
3.40 p.m.	0.72	0.16
	0.92	0.20
4 p.m.	0.13	0.09
	0.32	0.17

Experiment 26 (contd.)
Dog 26
21.3.56

	Time	Lymph mg. %	Blood mg. %
	11 p.m.	0.23	0.09
		0.32	0.15
22.3.56	11 a.m.	0.39	0.12
		0.65	0.32
	12.25 p.m.	Obstruct C.B.D. Tube	
	1.25 p.m.	0.42	0.18
		0.65	0.37
	2.25 p.m.	0.44	0.19
		0.62	0.32
	3.25 p.m.	0.51	0.28
		0.72	0.37
	4.25 p.m.	0.44	0.21
		0.77	0.30
	5.25 p.m.	0.42	0.24
		0.82	0.30
	6.25 p.m.	0.42	0.19
		0.80	0.30
23.3.56	12.25 a.m.	0.28	0.21
		0.77	0.42
	10 a.m.	0.67	0.66
		0.92	0.85
	12.25 p.m.	1.08	1.11
		1.37	1.37
	4.25 p.m.	1.05	1.11
		1.40	1.42
24.3.56	10 a.m.	1.62	1.99
		2.27	2.50
	4.25 p.m.	1.76	2.09
		2.35	2.85

BILIRUBIN (Acute G.B. out)

Experiment 27 Op. finished 10 a.m. G.B. out
Dog 27 C.B.D. Tube
15.3.56 Liver Lymph
Can. Portal Vein

	Time	Lymph mg. %	Blood (Portal Vein) mg. %
	12.15 p.m.	1.71	0.03
		1.95	0.04
	12.40 p.m.	0.66	0.09
		1.35	-
	1 p.m.	0.93	0.10
		1.15	-
	1.10 p.m. Obstruct C.B.D. Tube		
	1.20 p.m.	1.42	0.11
		1.7	-
	1.40 p.m.	3.0	0.14
		3.9	-
	2 p.m.	3.61	0.18
		4.2	-
	2.20 p.m.	4.41	0.19
		5.25	-
	2.40 p.m.	5.25	0.21
		5.62	-
	3 p.m.	6.66	0.26
		8.02	-
	3.10 p.m. Release C.B.D. Tube		
	3.40 p.m.	4.08	0.19
		7.27	-
	4 p.m.	3.98	0.24
		4.75	-
	4.20 p.m.	3.04	0.16
		3.55	-
16.3.56	4 p.m.	0.14	0.06
		0.31	-

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Experiment 27 (contd)
Dog 27

	Time	Lymph mg. %	Blood mg. %
16.3.56	4 p.m. Obstruct C.B.D. Tube		
	4 p.m.- 8 a.m. less than	0.5	-
17.3.56	12 noon	1.00	0.90
		1.20	1.10
	2 p.m.	1.05	1.22
		1.32	1.42
	4 p.m.	1.41	1.50
		1.77	2.15
	6 p.m.	1.71	1.78
		2.20	2.27
18.3.56	2 p.m.	2.15	3.13
		2.50	3.62

COMBINED STUDY (Acute G.B. out rec.)

Experiment 28 Op. finished 10 a.m. G.B. out
Dog 28 C.B.D. Tube
10.8.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
		Bilirubin	
	1 p.m.	0.33	0.09
		0.35	0.1
	1 p.m. Obstruct C.B.D. Tube		
	2 p.m.	0.48	0.07
		0.62	0.12
	3 p.m.	1.07	-
		1.25	-
	4 p.m.	4.7	0.15
		5.37	0.25
	4 p.m. Release C.B.D. Tube		
	5 p.m.	0.85	-
		0.87	-
	6 p.m.	0.24	0.07
		0.75	0.19
11.8.56	9 a.m.	0.24	0.09
		0.65	0.18
	9 a.m. Obstruct C.B.D. Tube		
	10 a.m.	0.39	-
		0.75	-
	11 a.m.	0.56	-
		1.05	-
	12 noon	0.42	0.19
		0.87	0.33
	1 p.m.	0.24	-
		0.62	-
	3 p.m.	0.33	0.33
		0.5	0.54
	5 p.m.	0.74	0.24
		0.87	0.44

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Experiment 28 (contd.)
Dog 28

	Time	Lymph mg. %	Blood mg. %
11.8.56	8 p.m.	0.66	0.49
		0.75	0.62
	12 midnight	0.70	0.76
		0.78	0.88
12.8.56	7 a.m.	0.99	1.15
		1.15	1.25
	5.30 p.m.	2.20	2.32
		2.55	2.62
	8.30 p.m.	2.39	2.47
		2.72	2.98
13.8.56	9 a.m.	2.97	3.21
		3.75	3.98
		B.S.P.	
12.8.56	6.30 p.m.	Inject 1 mg. per minute into leg vein in saline, 40 ml./hr.	
	7.30 p.m.	1.25	3.12
	8.30 p.m.	3.5	6.25
	9.30 p.m.	5.6	7.75
	9.30 p.m.	Stop injection	
13.8.56	6.30 p.m.	1.87	2.12

COMBINED STUDY (B.S.P. after obstr.)

Experiment 29 Op. finished 10 a.m. G.B. out
Dog 29 C.B.D. Tube
23.8.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
		Bilirubin	
	12 noon Obstruct C.B.D. Tube		
	2 p.m.	2.44	-
		3.62	-
	3 p.m.	3.05	0.09
		3.40	0.15
	4 p.m.	2.39	0.49
		2.75	0.62
	4 p.m. Release C.B.D. Tube		
	5 p.m.	0.90	0.38
		1.00	0.46
24.8.56	10 a.m.	0.89	0.38
		1.15	0.54
	10 a.m. Re-obstruct C.B.D. Tube		
	2 p.m.	0.49	0.23
		0.62	0.62
	4 p.m.	0.72	0.56
		0.92	0.84
	8 p.m.	0.96	0.78
		1.18	1.08
	12 midnight	1.04	1.12
		1.26	1.48
25.8.56	10 a.m.	1.32	2.22
		1.80	2.50

B.S.P. /

Experiment 29 (contd.)
Dog 29

Time		Lymph mg. %	Blood mg. %
		B.S.P.	
23.8.56	12 noon	Obstruct C.B.D. Tube	
	2 p.m.	Inject 1 mg. per minute into leg vein in saline, 40 ml./hr.	
	3 p.m.	2.92	2.75
	4 p.m.	8.87	5.87
	4 p.m.	Release C.B.D. Tube.	Stop injection.
	5 p.m.	3.50	1.80
	B.S.P. in bile after release		
		1st 0.5 ml.	25.0 mg.%
		2nd 0.5 ml.	105.0 mg.%
	4.01 p.m.	3rd 0.5 ml.	157.5 mg.%

COMBINED STUDY (Acute G.B. out rec.)

Experiment 30 Op. finished 10 a.m. G.B. out
Dog 30 C.B.D. Tube
13.8.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
		Bilirubin	
	11.30 a.m.	0.09	0.06
		0.10	0.07
	11.30 a.m. Obstruct C.B.D. Tube		
	1.30 p.m.	5.19	0.29
		5.5	0.5
	3.30 p.m.	7.75	-
		9.0	-
	4.30 p.m.	6.02	0.74
		6.75	0.75
	4.30 p.m. Release C.B.D. Tube		
14.8.56	11.30 a.m. Obstruct C.B.D. Tube		
	11.30 a.m.	0.56	0.16
		0.87	0.18
	2.30 p.m.	0.41	0.33
		0.76	0.4
	3.30 p.m.	0.59	0.41
		0.85	0.45
	4.30 p.m.	0.52	0.41
		0.75	0.60
	5.30 p.m.	0.57	0.42
		0.80	0.62
	12 midnight	0.41	0.74
		1.12	1.25
15.8.56	8 a.m.	1.40	1.89
		1.75	2.12
	2.30 p.m.	2.06	2.22
		2.62	2.87

Experiment 30 (contd.)
Dog 30

Time		Lymph mg. %	Blood mg. %
		B.S.P.	
13.8.56	11.30 a.m.	Obstruct C.B.D. Tube	
	1.30 p.m.	Inject 1 mg. per minute into leg vein in saline, 40 ml./hr.	
	2 p.m.	1.4	0.55
	2.30 p.m.	5.75	2.12
	3.30 p.m.	6.75	4.5
	4.30 p.m.	8.25	6.62
	4.30 p.m.	Release C.B.D. Tube. Stop injection.	
	Bile conc. B.S.P. after release		
		1st 0.5 ml. ..... 75 mg.%	
		2nd 0.5 ml. ..... 155 mg.%	
		3rd 0.5 ml. ..... 180 mg.%	
14.8.56	11.30 a.m.	0	0
	11.30 a.m.	Bile conc. B.S.P. 50 mg.%	
	11.30 a.m.	Obstruct C.B.D. Tube	
	2.30 p.m.	0	0
	2.30 p.m.	Inject 1 mg. per minute into leg vein in saline, 40 ml./hr.	
	3 p.m.	0.37	1.87
	3.30 p.m.	1.37	2.75
	4.30 p.m.	3.87	4.62
	5.30 p.m.	4.62	6.75
	5.30 p.m.	Stop injection.	

BILIRUBIN (Chronic G.B. out)

Experiment 31 — Op. finished 11 a.m. — G.B. out 22.3.56
Dog 31 — Six-day obstruction. — Lig. & Div. C.B.D. 22.3.56
28.3.56 — Liver Lymph 28.3.56

	Time	Lymph mg. %	Blood mg. %
	12 noon	3.30	5.55
		3.64	6.50
	2 p.m.	4.12	6.02
		5.00	6.64
	5 p.m.	4.05	6.27
		5.40	7.25
	7 p.m.	3.30	5.94
		3.75	7.00
29.3.56	10 a.m.	2.77	4.86
		3.50	5.75
	3 p.m.	3.63	5.32
		4.00	5.62
30.3.56	10 a.m.	4.04	5.19
		4.89	6.12
	5 p.m.	4.45	5.52
		5.01	6.37

BILIRUBIN (Chronic G.B. out)

Experiment 32
Dog 32
13.4.56

Op. finished 10 a.m.
Three-week obstruction.

G.B. out 22.3.56
Lig. & Div. C.B.D. 22.3.56
Liver Lymph 13.4.56

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	3.63	6.93
		4.50	8.25
	1 p.m.	4.12	6.93
		4.50	8.25
	6 p.m.	3.46	6.60
		4.25	8.25
14.4.56	10 a.m.	4.12	6.93
		4.50	7.50

BILIRUBIN (Chronic G.B. out)

Experiment 33
Dog 33
2.3.56

Op finished 10 a.m.
Five-week obstruction

G.B. out 24.1.56
Lig. & Div. C.B.D.
24.1.56
Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	8.8	15.84
		10.56	21.2
	4 p.m.	10.82	13.99
		11.6	14.96
3.3.56	11 a.m.	6.6	7.8
		9.24	10.2
	10 p.m.	6.47	9.64
		8.0	12.0

BILIRUBIN (Chronic)

Experiment 34
Dog 34
11.5.56

Twelve-day obstruction.
Cholecystectomy and
Ligation and Division C.B.D.
29.4.56

Specimen obtained at operation

Lymph mg. %	I.V.C. Blood mg. %
4.95	6.76
5.62	8.25

The dog's condition was poor and it was not allowed to recover from the anaesthetic.

BILIRUBIN (Chronic)

Experiment 35
Dog 35
16.5.56

Nineteen-day obstruction.
Cholecystectomy and
Ligation and Division C.B.D.
27.4.56

Specimen obtained at operation

Lymph mg. %	I.V.C. Blood mg. %
19.47	22.77
22.25	27.5

The dog's condition was poor and it was not allowed to recover from the anaesthetic.

BILIRUBIN (Chronic G.B. in)

Experiment 36 — Op. finished 10 a.m. — G.B. in
Dog 36 — Eight-day obstruction — Lig. & Div. C.B.D.
22.6.56 — 14.6.56
Liver Lymph

	Time	Lymph mg. %	Blood mg. %
	11 a.m.	4.60	7.25
		5.25	8.37
		Lymph catheter out	
23.5.56	10 a.m.	-	7.59
		-	8.87

Recanulation of Liver Lymphatic under Anaesthesia 27.6.56
(Thirteen-day obstruction)

	5.11	7.42
	5.80	8.0

BILIRUBIN (Chronic G.B. in)

Experiment 37 Op. finished 10 a.m. G.B. in
Dog 37 Four-day obstruction. Lig. & Div. C.B.D.
18.6.56 14.6.56

Time	Lymph mg. %	Blood mg. %
Pre-op.	-	5.52
	-	6.30
At op.	4.62	-
	5.2	-
Post-op.	5.5	6.03
	6.37	7.12
2 p.m.	Lymph catheter blocked	

BILIRUBIN (Chronic G.B. in)

Experiment 38 Dog 38 12.7.56	Nine-day obstruction. Specimen obtained at operation.	G.B. in Lig. & Div. C.B.D. 3.7.56 Liver Lymph

Lymph mg. %	Blood mg. %
2.5	3.1
3.0	3.55

Animal's condition poor. Sacrificed under anaesthesia.

B.S.P. (Acute Obstr.)

Experiment 39
Dog 39
1.3.56

G.B. out
C.B.D. Tube
Can. Portal Vein
Liver Lymph

Time	Lymph mg. %	Portal Vein mg. %	Bile mg. %
12 noon. Inject 1 mg. per minute into leg vein in 40 ml. saline			
12.25 p.m.	0.00	0.57	37.00
12.45 p.m.	0.80	0.72	277.50
1.05 p.m.	1.22	0.80	312.50
1.25 p.m.	1.30	Haemolysis	332.50
1.30 p.m. Obstruct C.B.D. Tube			
1.45 p.m.	-	1.00	
2.05 p.m.	2.45	-	
2.25 p.m.	2.50	1.67	
2.45 p.m.	-	1.95	
3.05 p.m.	4.50	2.00	
3.25 p.m.	4.70	2.40	
3.30 p.m. Release C.B.D. Tube			
3.45 p.m.	4.37	2.07	270.00
4.05 p.m.	3.72	2.00	360.00
4.25 p.m.	2.85	1.82	420.00
4.45 p.m.	2.65	1.52	465.00
4.50 p.m. Stop injection			
2.3.56 10 a.m. Inject 1 mg. per minute into leg vein			
10.25 a.m.	0.00	0.72	42.50
10.45 a.m.	0.20	1.07	177.50
11.05 a.m.	0.50	1.20	312.50

11.25 /

Experiment 39 (contd)
Dog 39

	Time	Lymph mg. %	Portal Vein mg. %	Bile mg. %
2.3.56	11.25 a.m.	0.72	1.57	387.50
	11.30 a.m.	Obstruct C.B.D. Tube		
	11.45 a.m.	1.25	2.00	
	12.05 p.m.	1.70	3.10	
	12.25 p.m.	2.62	3.82	
	12.45 p.m.	3.32	4.27	
	1.05 p.m.	3.60	4.75	
	1.25 p.m.	4.40	5.30	
	1.45 p.m.	4.50	5.75	
	2.05 p.m.	4.90	5.92	
	2.25 p.m.	5.15	6.27	
	2.30 p.m.	Stop injection.		

COMBINED STUDY (Acute G.B. out rec.)

Experiment 40 Op. finished 10 a.m. G.B. out
Dog 40 C.B.D. Tube
7.8.56 Liver Lymph

	Time	Lymph mg. %	Blood mg. %
		Bilirubin	
	1 p.m.	0.49	0.03
		0.5	0.04
	1 p.m.	Obstruct C.B.D. Tube	
	2 p.m.	0.99	0.099
		1.15	1.0
	3 p.m.	4.95	0.66
		5.75	1.2
	4 p.m.	6.95	0.9
		8.12	1.25
	4 p.m.	Release C.B.D. Tube	
	5 p.m.	2.97	0.13
		3.62	0.15
	6 p.m.	0.75	0.02
		1.11	0.15
8.8.56	11 a.m.	0.57	0.21
		0.87	0.4
	11 a.m.	Obstruct C.B.D. Tube	
	12 noon	0.66	0.29
		1.25	0.44
	1 p.m.	0.99	0.29
		1.65	0.5
	2 p.m.	0.99	0.31
		1.65	0.52
	5 p.m.	1.40	0.75
		1.62	0.85
	8 p.m.	1.70	1.02
		2.1	1.3
	12 midnight	2.31	2.64
		2.55	2.75

9.8.56 /

Experiment 40 (contd.)
Dog 40

	Time	Lymph mg. %	Blood mg. %
		Bilirubin	
9.8.56	8 a.m.	3.30	3.38
		3.37	3.80
	12 noon	3.06	3.54
		3.5	3.75
		B.S.P.	
7.8.56	11 a.m.	Inject 0.5 mg. per minute into leg vein in saline, 5 ml./hr.	
	12.30 p.m.	1.12	0.75
	1 p.m.	1.25	0.87
	1 p.m.	Bile conc. B.S.P. 465 mg.%	
	1 p.m.	Obstruct C.B.D. Tube	
	2 p.m.	3.75	1.5
	3 p.m.	7.75	3.25
	4 p.m.	11.0	3.87
	4 p.m.	Release C.B.D. Tube.	Stop injection.
	5 p.m.	7.0	0.35
	6 p.m.	1.87	0.2
8.8.56	9 a.m.	Inject o.5 mg. per minute into leg vein in saline, 5 ml./hr.	
	10.30 a.m.	0.68	0.75
	11 a.m.	0.92	1.0
	11 a.m.	Bile conc. B.S.P. 1500 mg.%	
	11 a.m.	Obstruct C.B.D. Tube	
	12 noon	1.75	2.5
	1 p.m.	3.75	3.62
	2 p.m.	4.12	4.12
	2 p.m.	Stop injection.	

COMBINED STUDY (Acute G.B. out rec.)

Experiment 41. Op. finished 11 a.m. G.B. out
Dog 41. C.B.D. tube
6.8.56. Liver lymph

Time	Lymph mg.%	Blood mg.%
	Bilirubin	
2 p.m.	0.28	0.024
	0.45	0.075
2 p.m.	Obstruct C.B.D. Tube	
3 p.m.	0.8	0.08
	1.25	0.14
4 p.m.	1.65	0.16
	2.25	0.22
5 p.m.	4.87	0.19
	6.3	0.38
5 p.m.	Release C.B.D. Tube	
6 p.m.	0.03	0.26
	0.05	0.34
7 p.m.	0.04	0.08
	0.05	0.12
	B.S.P.	
6.8.56. 12 noon	Inject 0.5 mg. per minute into leg vein in saline, 40 ml./hr.	
1.30 p.m.	1.37	0.75
	2.0	0.87
2 p.m.	Bile conc. of B.S.P. 530 mg.% Obstruct C.B.D. Tube	
3 p.m.	2.37	1.12
4 p.m.	5.25	2.25
5 p.m.	6.87	3.25
5 p.m.	Release C.B.D. Tube & Stop Injection	

COMBINED STUDY (B.S.P. after Obstr.)

Experiment 42. Op. finished 10 a.m. G.B. out
Dog 42. Obstr. C.B.D. Tube 10 a.m. C.B.D. Tube
14.9.56. Liver lymph

	Time	Lymph mg.%	Blood mg.%
		Bilirubin Total	
	2 p.m.	7.5	-
	3 p.m.	8.75	-
	4 p.m.	4.5	-
	5 p.m.	6.54	-
	5 p.m.	Release C.B.D. Tube	
		B.S.P.	
14.9.56.	2 p.m.	Inject 1.0 mg. per minute into leg vein in saline, 5 ml./hr.	
	3 p.m.	17.25	3.62
	4 p.m.	20.0	6.0
	5 p.m.	29.5	7.87
	5 p.m.	Stop injection.	Release C.B.D. Tube

B.S.P. in bile after release

	mg.%
1st 0.5 ml.....	0.5
2nd 0.5 ml.....	22.0
3rd 0.5 ml.	280.0
Next 2.0 ml. ..	1180.0
Next 2.0 ml. ..	1180.0

COMBINED STUDY (Chronic G.B. out)

Experiment 43. Op. finished 10 a.m. G.B. out 17.7.56.
Dog 43. 7 Day obstruction Lig. & Div. C.B.D.
24.7.56. 17.7.56.
Liver lymph

	Time	Lymph mg.%	Blood mg.%
		Bilirubin	
	11 a.m.	4.12	5.77
		4.62	6.35
	2 p.m.	2.8	-
		3.2	-
	5 p.m.	3.36	4.75
		3.65	5.3
25.7.56.	10 a.m.	3.95	4.92
		4.75	5.75
	2 p.m.	4.04	5.19
		5.0	6.12
		B.S.P.	
24.7.56.	2 p.m.	Inject 0.5 mg. per minute into leg vein in saline, 40 ml./hr.	
	2.30 p.m.	0.62	1.25
	3 p.m.	1.5	2.15
	4 p.m.	2.12	4.62
	5 p.m.	3.62	5.5
		Stop B.S.P. injection	
	5.30 p.m.	3.5	-
	6 p.m.	3.37	-
	8 p.m.	2.12	3.3
25.7.56.	10 a.m.	1.0	1.6
	2 p.m.	0.5	1.0

COMBINED STUDY (Chronic G.B. out)

Experiment 44. Op finished 10 a.m. G.B. out 17.7.56.
Dog 44. 10 day obstruction Lig. & Div. C.B.D. 17.7.56.
27.7.56. Liver lymph

	Time	Lymph mg.%	Blood mg.%
		Bilirubin	
	10 a.m.	3.05	4.14
		3.5	4.5
	2 p.m.	3.54	4.45
		4.12	5.1
	4 p.m.	3.13	3.96
		3.87	4.5
	5 p.m.	3.63	3.87
		4.25	4.5
	7 p.m.	3.79	4.05
		4.40	4.90
	8 p.m.	3.79	4.05
		4.40	4.90
	12 midnight	3.71	4.35
		4.5	5.15
28.7.56.	10 a.m.	3.89	4.70
		4.75	5.87
	2 p.m.	3.92	4.78
		4.80	5.80
		B.S.P.	
27.7.56.	2 p.m.	Inject 0.5 mg. per minute into leg vein in saline, 40 ml./hr.	
	3 p.m.	1.5	3.0
	4 p.m.	2.5	3.9
	5 p.m.	3.37	4.87

Stop /

Experiment 44 (contd.)
Dog 44.

	Time	Lymph mg.%	Blood mg.%
	Stop B.S.P. Injection		
	5.30 p.m.	3.75	-
	6 p.m.	3.75	3.75
	6.30 p.m.	3.12	3.12
	7 p.m.	3.0	3.0
	8 p.m.	2.42	2.37
	9 p.m.	2.0	1.87
	12 midnight	1.5	1.37
28.7.56.	10 a.m.	0.875	1.0
	2 p.m.	0.72	0.94

COMBINED STUDY (B.S.P. After Obstr.)

Experiment 45. Op finished 10 a.m. G.B. out
Dog 45. Lig. & Div. Ducts 10 a.m. Lig. & Div.C.B.D.
4.9.56. Liver lymph

Time	Lymph mg.%	Blood mg.%
	Bilirubin	
11 a.m.	2.02	0.10
	2.36	0.13
2 p.m.	9.40	0.07
	11.12	0.13
3 p.m.	8.83	-
	9.12	-
4 p.m.	8.95	-
	9.5	-
5 p.m.	8.0	0.74
	8.75	0.87
6 p.m.	7.92	-
	8.75	-
7 p.m.	9.73	-
	11.25	-
8 p.m.	13.05	-
	14.37	-
	B.S.P.	
2 p.m.	Inject 1.0 mg. per minute into leg vein in saline, 40 ml./hr.	
3 p.m.	5.95	3.07
4 p.m.	9.62	5.25
5 p.m.	14.75	7.0
5 p.m.	Stop injection	
6 p.m.	8.75	-
7 p.m.	6.87	-
8 p.m.	6.25	-

COMBINED STUDY (Saline control first day, obstr.)
(B.S.P. after 24 hr. of obstr.)

Experiment 46.
Dog 46.
10.9.56.

Op. finished 10 a.m.
Obstr. C.B.D. tube 10 a.m.

G.B. out
C.B.D. Tube
Liver lymph

Time	Lymph mg.%	Blood mg.%
	Bilirubin(Total)	
2 p.m.	Inject 40 ml. saline per hr.	
2 p.m.	8.0	-
3 p.m.	16.5	-
4 p.m.	18.7	-
5 p.m.	Stop injection	
5 p.m.	19.6	-
6 p.m.	14.75	-
10 p.m.	4.25	-
11.9.56. 10 a.m.	3.25	2.87
5 p.m.	2.62	3.15
	B.S.P.	
11.9.56. 2 p.m.	Inject 1.0 mg. per minute into leg vein in saline, 40 ml./hr.	
3 p.m.	2.25	5.0
4 p.m.	3.62	7.5
5 p.m.	6.0	8.75
5 p.m.	Stop injection. Release C.B.D. Tube	
	B.S.P. in bile after release.	

1st /

Experiment 46 (contd.)
Dog 46.

Time					Colour of Bile	Lymph Conc.
	1st	0.5 ml..	0	mg.%	Dark green	
	2nd	0.5 ml..	0	"	Green	
	3rd	0.5 ml..	0.5	"	White	
	4th	0.5 ml..	1.75	"	"	
	5th	0.5 ml..	1.62	"	"	
5.02 p.m.	6th	0.5 ml..	0.75	"	"	
5.22 p.m.	Next	0.8 ml..	1.5	"	Faint yellow	4.75 mg.%
5.45 p.m.	Next	0.9 ml..	1.75	"	Faint yellow	
		4.7				
6 p.m.	Next	2.2 ml.	12.25	"		7.25 "
6.15 p.m.	Next	1.4 ml.	14.25	"		5.0 "

COMBINED STUDY (Saline control first day, obstr.)
B.S.P. after 24 hr. of obstr.

Experiment 47.
Dog 47.
6.9.56.

Op. finished 10 a.m.
Obstr. C.B.D. Tube 10 a.m.

G.B. out
C.B.D. Tube
Liver lymph

Time		Lymph mg.% Bilirubin	Blood mg.%
	11 a.m.	1.75	0.12
		1.98	0.14
	2 p.m.	4.70	-
		5.37	-
	2 p.m.	Inject 40 ml. saline per hr. into leg vein	
	3 p.m.	5.44	-
		6.25	-
	4 p.m.	6.75	-
		7.75	-
	5 p.m.	7.42	-
		8.37	-
	5 p.m.	Stop injection.	
	7 p.m.	7.59	-
		8.75	-
	9 p.m.	9.42	-
		10.65	-
7.9.56.	10 a.m.	4.78	4.42
		5.32	4.62

B.S.P.

7.9.56.	10 a.m.	Inject 1.0 mg. per minute into leg vein in saline, 40 ml./hr.	
	11 a.m.	4.64	7.37
	12 noon.	6.87	11.0
	1 p.m.	10.0	13.0
	1 p.m.	Stop injection. Release C.B.D. Tube	
		B.S.P. in bile after release	

1st /

Experiment 47 (contd.)
Dog 47.

Time				Colour of Bile	Lymph con.
	1st 0.5 ml..	0	mg.%	Dark green	
	2nd 0.5 ml..	0	"	White	
	3rd 0.5 ml..	0.87	"	"	
	4th 0.5 ml..	0.75	"	"	
	5th 0.5 ml..	0.82	"	"	
1.02 p.m.	6th 0.5 ml..	1.02	"	"	
1.45 p.m.	Next 2.1 ml..	1.64	mg.%	Faint yellow	6.52 mg.%
	5.1				

COMBINED STUDY (B.S.P. after 24 hr. of obstr.)

Experiment 48 Op. finished 10 a.m. G.B. out
Dog 48 Obstr. C.B.D. Tube 10 a.m. C.B.D. Tube
12.9.56. Liver lymph

	Time	Lymph mg.%	Blood mg.%
		Bilirubin Total	
	4 p.m.	17.80	-
	6 p.m.	16.74	-
13.9.56.	10 a.m.	5.35	3.25
	2 p.m.	3.75	3.5
	5 p.m.	3.42	3.69
	5 p.m.	Release C.B.D. Tube	
	7 p.m.	2.89	2.87
		B.S.P.	
13.9.56.	2 p.m.	Inject 1.0 mg. per minute into leg vein in saline, 40 ml./hr.	
	3 p.m.	1.37	4.87
	4 p.m.	5.62	8.12
	5 p.m.	8.25	11.0
	5 p.m.	Release C.B.D. Tube. Stop injection.	
		B.S.P. in bile after release.	

1st /

Experiment 48 (contd.)
Dog 48.

Time				Colour of Bile	Lymph mg.%	Blood mg.%
	1st	0.5 ml..	0 mg.%	Green		
	2nd	0.5 ml..	0 "	White		
	3rd	0.5 ml..	0 "	"		
	4th	0.5 ml..	0 "	"		
	5th	0.5 ml..	0 "	"		
5.02 p.m.	6th	0.5 ml..	0 "	"		
5.10 p.m.	7th	0.5 ml..	0 "	"		
5.35 p.m.	8th	0.5 ml..	0 "	"		
6.05 p.m.	9th	0.5 ml..	0 "	"	7.87	9.12
6.25 p.m.	10th	0.5 ml..	0.37 "	Faint yellow	5.62	5.62
		5.0				
7 p.m.	11th	0.5 ml..	2.87		5.25	5.0
9 p.m.	12th	0.5 ml..	3.62			
	Next	2.0 ml..	4.75			

BILIRUBIN (Bile after release at 24 hrs.)

Experiment 49
Dog 49
20.9.56

Op. finished 10 a.m.
Obstruct C.B.D. Tube
10 a.m., 19.9.56

G.B. out
19.9.56
C.B.D. Tube
19.9.56

Time	Sample	mg.%	Colour of Bile	Blood mg. %
2 p.m. Release C.B.D.	1st 0.5 ml....	4.25	Dark green	
	2nd 0.5 ml....	0.165	Light green	
	3rd 0.5 ml....	0.0	White	
	4th 0.5 ml....	0.0	White	
2.02 p.m.	5th 0.5 ml....	0.5	White	
2.10 p.m.	6th 0.5 ml....	0.5	White	
2.50 p.m.	7th 0.5 ml....	2.25	Light yellow	
3.30 p.m.	Next 1.5 ml....	4.25	Yellow	4.32 4.5

BILIRUBIN (Bile after release at 24 hrs.)

Experiment 50 Dog 50 20.9.56	Op. finished 11 a.m. Obstruct C.B. Tube 11 a.m. 19.9.56	G.B. out 19.9.56 C.B.D. Tube 19.9.56

Time	Sample	Colour of Bile	Blood mg. %
	mg.%		
2 p.m. Release C.B.D. tube	1st 0.5 ml....14.0	Dark green	
	2nd 0.5 ml.... 5.5	Green	
	3rd 0.5 ml.... 3.5	Light green	
	4th 0.5 ml.... 1.75	Light green	
	5th 0.5 ml.... 1.75	Light green	
2.02 p.m.	6th 0.5 ml.... 3.5	Light green	
2.04 p.m.	7th 0.5 ml.... 4.0	Green	
2.08 p.m.	8th 0.5 ml.... 4.5	Green	
2.14 p.m.	9th 0.5 ml.... 4.0	Green	
2.22 p.m.	10th 0.5 ml....17.5	Dark green	
2.28 p.m.	11th 0.5 ml....22.0	Dark green	
2.37 p.m.	12th 0.5 ml....38.0	Yellow	3.1 4.2

BILIRUBIN (Bile after release at 24 hrs.)

Experiment 51
Dog 51
25.9.56

Obstr. C.B.D. tube 12 noon on 24.9.56

G.B. out 22.9.56
C.B.D. tube 22.9.56

Time	Sample	mg.%	Colour of Bile	Blood mg. %
2 p.m. Release C.B.D. tube	1st 0.5 ml....	16.5	Dark green	
	2nd 0.5 ml....	1.25	Light green	
	3rd 0.5 ml....	0.0	White	
	4th 0.5 ml....	0.0	White	
2.02 p.m.	5th 0.5 ml....	0.0	White	
	6th 0.5 ml....	0.0	White	
2.10 p.m.	7th 0.5 ml....	0.0	White	
	8th 0.5 ml....	0.0	White	
2.15 p.m.	9th 0.5 ml....	0.0	White	
	10th 0.5 ml....	0.0	White	
2.20 p.m.	11th 0.5 ml....	0.0	White	
	12th 0.5 ml....	0.0	White	
2.28 p.m.	13th 1.0 ml....	0.5	Light yellow	2.3

BILIRUBIN (Bile after release at 24 hrs.)

Experiment 52
Dog 52
28.9.56

Obstr. C.B.D. tube 12 noon on 27.9.56

G.B. out 27.9.56
C.B.D. Tube 27.9.56

Time	Sample		Colour of Bile	Blood mg. %
		mg.%		
2 p.m. Release C.B.D.	1st 0.5 ml....	12.25	Dark green	
	2nd 0.5 ml....	2.40	Light green	
	3rd 0.5 ml....	0.6	White	
	4th 0.5 ml....	0.84	White	
2.04 p.m.	5th 0.5 ml....	0.82	White	
	6th 0.5 ml....	0.6	White	
2.23 p.m.	Next 2.0 ml....	1.26	Light yellow	2.88

BILIRUBIN (Acute Obstruction)

Experiment A Op finished 12 a.m. Lig. & Div.C.B.D.
Rat A (Weight 212 g.) Liver Lymph
5.6.56

	Time	Lymph mg. %	Blood mg. %
	12- 4 p.m.	0.82 1.0	-
	4- 6 p.m.	1.40 1.62	-
	6-10 p.m.	1.84 2.0	-
	10-12 mn.	2.47 2.75	-
6.6.56	12- 8 a.m.	2.47 3.0	-
	8-12 a.m.	1.98 2.25	-
	12- 2 p.m.	1.73 2.12	1.65 2.09

BILIRUBIN (Acute Obstruction)

Experiment B Op. finished 11 a.m. Lig. & Div.C.B.D.
Rat B (Weight 265 g.) Liver Lymph
27.8.56

	Time	Lymph mg. %	Blood mg. %
	12- 4 p.m.	0.68	-
		0.94	
	4- 8 p.m.	1.20	-
		1.38	
	8-12 mn.	1.92	-
		2.04	
28.8.56	12- 8 a.m.	2.26	-
		2.44	
	8-12 a.m.	2.22	2.16
		2.48	2.28

BILIRUBIN (Acute Obstruction)

Experiment C Op. finished 12 noon Lig. & Div.C.B.D.
Rat C (Weight 225 g.) Liver Lymph
27.8.56

	Time	Lymph mg. %	Blood mg. %
	12- 4 p.m.	0.80 1.34	-
	4- 8 p.m.	1.36 1.90	-
	8-12 mn.	2.16 2.58	-
28.8.56	12- 8 a.m.	2.44 2.80	-
	8-12 a.m.	2.62 2.88	2.47 2.70

BILIRUBIN (Acute Obstruction)

Experiment D Op. finished 10 a.m. Lig. & Div. C.B.D.
Rat D (Weight 270 g.) Liver Lymph
1.6.56

	Time	Lymph mg. %	Blood mg. %
	10-11 a.m.	0.90 1.22	-
	11-12 a.m.	1.48 1.62	-
	12- 1 p.m.	1.56 1.75	-
	1- 2 p.m.	1.15 1.37	-
	2- 3 p.m.	1.15 1.37	-
	3- 4 p.m.	1.15 1.37	-
	4-12 mn.	1.71 1.95	-
2.6.56	12-10 a.m.	2.34 2.80	-
3.6.56	10-10 a.m.	2.86 3.60	-
	10-12 a.m.	4.62 5.12	4.78 6.30

BILIRUBIN (Acute Obstruction)

Experiment E Op. finished 11 a.m. Lig. & Div.C.B.D.
Rat E (Weight 260 g.) Liver Lymph
1.6.56

	Time	Lymph mg. %	Blood mg. %
	11-12 a.m.	0.66 0.75	-
	12-1 p.m.	0.66 0.75	-
	1-2 p.m.	0.49 0.75	-
	2-3 p.m.	0.66 0.75	-
	3-4 p.m.	0.66 0.90	-
	4-12 mn.	1.65 2.00	-
2.6.56	12-11 a.m.	1.90 2.30	-
3.6.56	11-11 a.m.	2.64 3.45	-
	11-1 p.m.	4.62 5.50	4.98 5.75

BILIRUBIN (Acute obstruction)

Experiment F Op. finished 12 a.m. Lig. & Div.C.B.D.
Rat F (Weight 250 g.) Liver Lymph
1.6.56

	Time	Lymph mg. %	Blood mg. %
	12- 1 p.m.	0.82	-
		1.00	
	1-2 p.m.	0.75	-
		0.75	
	2-3 p.m.	1.10	-
		1.30	
	3-4 p.m.	1.43	-
		2.10	
	4-5 p.m.	1.43	-
		1.45	
	5-12 mn.	2.14	-
		2.14	
2.6.56	12-12 a.m.	2.97	-
		3.20	
3.6.56	12-12 a.m.	3.16	-
		4.20	
	12-2 p.m.	5.11	6.54
		6.70	7.25

BILIRUBIN (Chronic Obstruction)

Experiment G Two-day obstruction. Lig. & Div. C.B.D.
Rat G (Weight 245 g.) 25.8.56
27.8.56 Liver Lymph
27.8.56

Lymph mg. %	Blood mg. %
5.03	5.69
5.87	6.50

BILIRUBIN (Chronic Obstruction)

Experiment H — Three-day obstruction. Lig. & Div. C.B.D. 25.8.56
Rat H (Weight 250 g.) — Liver Lymph 27.8.56
28.8.56

Lymph mg. %	Blood mg. %
4.75	4.88
5.37	5.87

BILIRUBIN (Chronic Obstruction)

Experiment I — Three-day obstruction. Lig. & Div. C.B.D. 21.8.56
Rat I (Weight 255 g.) Liver Lymph 24.8.56
24.8.56

Lymph mg. %	Blood mg. %
3.96	3.79
4.00	4.00

BILIRUBIN (Chronic Obstruction

Experiment J Four-day obstruction. Lig. & Div.C.B.D. 17.8.56
Rat J (Weight 240 g.) Liver Lymph 20.8.56
21.8.56

Lymph mg. %	Blood mg. %
3.84	4.45
4.25	5.00

BILIRUBIN (Chronic Obstruction)

Experiment K Five-day obstruction. Lig. & Div. C.B.D. 17.8.56
Rat K (Weight 270 g.) Liver Lymph 21.8.56
22.8.56

Lymph mg. %	Blood mg. %
5.61	6.43
5.75	7.00

BILIRUBIN (Chronic Obstruction)

Experiment L — Ten-day obstruction. — Lig. & Div. C.B.D. 29.8.56
Rat L (Weight 248 g.) — Liver Lymph 7.9.56
8.9.56

Lymph mg. %	Blood mg. %
4.18	4.25
4.25	4.35

APPENDIX A

SOME OBSERVATIONS ON THE ANATOMY OF THE HILAR LYMPHATICS FROM THE CANINE LIVER

Although descriptions of tissue spaces and lymphatic vessels within the liver itself have not infrequently been reported (Bollman, 1950; Dissé, 1890; Johnson and Mann, 1950 1901,+ Mall, 1906; ~~Malloy and Evelyn, 1937~~) little factual information is to be found in the literature about the anatomy of the extrahepatic portion of this system.

No doubt the collapsed, thread-like appearance of these structures in the morbid preparation has discouraged study, but it seems equally likely that the lack of practical physiological methods for canulating them has diverted attention elsewhere.

Interest in study of the biochemistry of liver lymph has increased steadily during the last decade. Suitable techniques for canulation of the liver lymphatic trunks have since been described in the dog (Grindlay et al., 1948, 1950), rat (Bollman et al, 1948) and, more recently, in the cat (Morris, 1956). A better understanding of the anatomy of the hilar lymphatics /

lymphatics, apart from being desirable on general grounds, now seems likely to assist in the expansion of the use and scope of these techniques and to provide a more rational basis for the evaluation of results obtained therefrom.

Pronounced engorgement of its associated lymphatics follows ligation of the main efferent trunk from the liver, particularly if ether anaesthesia which, of itself, promotes the flow of hepatic lymph (Drinker and Yoffey, 1941; Fisher et al., 1956; McAllister, 1937; Stewart, unpublished work) is being used. This greatly facilitates their recognition.

It is therefore with such observationson the dynamic anatomy of the hilar lymphatic system of the canine liver that the present appendix is concerned.

Methods and Materials

Consecutive examinations were made of the hilar lymphatics in 100 healthy mongrel dogs. The preparations were used subsequently for the study set out in this thesis.

As described above the animals were anaesthetised with ether and, through a thoraco-abdominal incision in the right tenth interspace, splitting the diaphragm radially, the hilus of the liver was exposed. The main efferent lymphatic trunk was ligated as far distally as possible.

Careful /

Careful account was taken of the anatomy before and after this ligation and schematic drawings and notes of the part were made on each occasion from which the data now to be presented have been extracted.

The Anatomical Findings

General Description of the Lymphatic Pathways from the Hilus of the Canine Liver

Lymph leaves the canine liver by two routes (Bollman, 1950); the major of these, the hilar route, is the object of this study. The minor pathway is via the hepatic veins to the thoracic duct. Careful examination of all the preparations described herein supported this view and no evidence of lymphatic plexi on the abdominal or thoracic surfaces of the diaphragm, such as are described in man (Cunningham, 1951) was found.

The field exposed for examination in these preparations was in the shape of a quadrilateral (Fig. 1). It was limited cephalad by the right free edge of the lesser omentum with the bile ducts, portal vein and coeliac axis; caudally, by the superior mesenteric artery; ventrally, with the duodenal mesentery on stretch as at operation (Grindlay, 1950) by the superior mesenteric vein, the termination of the splenic /

splenic vein and the lymphatic elements enclosed in the angle between them; dorsally, by the inferior vena cava (Fig. 1).

Afferent lymphatics from the hilus appear to follow one of two routes.

(a) The Main Hepatic System.- Lymph from the right lobes of the liver leaves the right side of the porta hepatis in from two to six afferent vessels. These run along the ventral aspect of the portal vein and turn caudally just before its origin into the hepatic gland, which can usually be seen through the peritoneum of the duodenal mesentery as it lies in the angle made between the coeliac artery and the portal and superior mesenteric veins. The gland is not normally in contact with either of these vessels. Its long axis lies on the diagonal of this quadrilateral passing to that corner formed by the inferior vena cava and the superior mesenteric artery. It occupies about one-third of this distance.

From this gland the main hepatic lymph trunk usually arises, to pass backwards along the remaining two-thirds of the diagonal and by the medial side of the inferior vena cava to join an ascending lumbar lymph trunk or the cysterna chyli itself.

(B) /

(B) The Accessory Hepatic System.- A second group of glands lies in the duodenal mesentery just ventral to the superior mesenteric vein and in the angle made at its junction with the splenic vein. To these glands the term accessory hepatic group will be applied in this description. They drain the remaining afferents leaving the hilus, drawing mainly on those from the left lobe which pass through the lesser omentum and over the termination of the splenic and origin of the portal veins to reach them. In every case one or two branches from radicles on the portal vein can be seen to interconnect with them.

From this group of glands separate efferent lymphatics pass backwards across the right side of the superior mesenteric vein to join the main hepatic trunk above its middle or, less frequently, they combine to form a discrete accessory hepatic lymph trunk to do this.

A more detailed account of some features of both systems follows:-

The Main Hepatic System

The Hepatic Gland and Related Anomalies.- The gland is usually single and this was the case in 92 per cent. of the animals examined. In 8 per cent. (Nos. 1, 5, 13, 15, 19, 57, 58, /

58, 85) two glands were present at the usual site, the additional gland occupying the middle third of the same diagonal.

Atopic lymph glands connected with this system were observed on two occasions (2.0 per cent.).

(1) A gland was present in the right free edge of the lesser omentum, cephalad to the coeliac artery and dorsal to the portal vein (No. 4).

(2) A gland was found cephalad to the splenic vein and medial to the portal vein (No. 82).

Abnormalities of the Hepatic Gland Itself.- These were observed on six occasions (6.0 per cent.).

(1) Abnormal peritoneal attachments:-

In two animals the gland was mobile and suspended in a peritoneal fold. One of these (No. 4) was attached at the usual site but lay superficial to the duodenal mesentery. In the other preparation (No. 60) the gland lay deep in the foramen of Winslow, behind the coeliac axis and could not be seen until displaced outwards.

(2) Abnormal fixation:-

On two occasions (Nos. 31, 78) the gland was connected by a bridge of adenoid tissue passing ventrally over the right side of the superior mesenteric vein to a gland in the accessory hepatic group (2.0 per cent.). It was firmly adherent to the superior mesenteric vein itself in another preparation /

preparation (No. 87).

(3) Abnormality of shape:-

The gland, which is usually bean-shaped, was found to be long, thin and tapered, reaching backwards almost to the inferior vena cava in one animal (No. 66). Consequently it gave off a very short main hepatic trunk.

The Main Hepatic Lymph Trunk and Related Anomalies.- The trunk is usually about one inch long and has the diameter of a match-stick when the animal being examined is under ether anaesthesia. It can often be seen to be constricted at one to three points where lymphatic valves are present within. These are the nearest valves in this system to the liver itself. In 37.0 per cent. of the animals examined however this trunk was either small or bifid or trifid. On one occasion in this series no main hepatic trunk was seen (No. 39).

Where the trunk seemed to be split, since two or three efferent trunksissued from the main hepatic gland, these finally came together to form a short stump-like vessel which entered the cysterna chyli. On one occasion (No. 70) the trunk was bifid only in its proximal half.

Abnormality /

Abnormality of Main Hepatic Trunk	Percentage Occurrence	Dog Numbers
Small	20	2, 3, 6, 11, 19, 20, 22, 29, 30, 31, 32, 41, 43, 49, 51, 54, 66, 78, 95, 97.
Bifid	14	15, 18, 23, 44, 56, 60, 61, 70, 80, 81, 82, 84, 85, 90.
Trifid	3	28, 71, 75.

On a further twelve occasions 12.0 per cent. (Nos. 16, 25, 33, 35, 36, 45, 46, 83, 87, 94, 96, 99) the main trunk was abnormally short. It did not attain normal size until joined by efferents from the accessory group which entered it at an unusually low level near the inferior vena cava.

The trunk exhibited a curious cavernous transformation in 5.0 per cent. (Nos. 17, 24, 45, 63, 77).

In 5.0 per cent. the main hepatic trunk was joined by the intestinal lymph trunk to form a common vessel just before it entered the cysterna chyli (Nos. 1, 9, 10, 21, 99).

Abnormalities in the course taken by the trunk.- These were seen in 5.0 per cent.

(1) It crossed the superior mesenteric artery and the left renal vein before turning dorsally into the receiving trunk /

trunk in 3.0 per cent. (Nos. 48, 81, 83).

(2) It crossed the superior mesenteric artery alone before turning dorsally in 1.0 per cent. (No. 70).

(3) It passed ventrally over the superior mesenteric vein in 1.0 per cent. (No. 91) to join an accessory hepatic trunk.

Some Anatomical Features Associated with Abnormalities of the Main Hepatic Lymph Trunk

Marked diminution in the size of the quadrilateral space through which the trunk passes is usually associated with shortening and underdevelopment. High origin of the superior mesenteric artery with or without low origin of the coeliac axis reduces the space lengthwise. This was observed in 35 of the animals examined (Nos. 2, 4, 6, 8, 15, 16, 17, 18, 19, 20, 25, 29, 31, 32, 36, 39, 41, 42, 43, 45, 51, 52, 60, 61, 66, 75, 77, 78, 80, 82, 84, 85, 90, 95, 97) and the trunk was poorly developed in 23 of these (Nos, 2, 6, 15, 18, 19, 20, 31, 32, 36, 39, 41, 45, 60, 61, 66, 75, 80, 82, 84, 85, 90, 95, 97). Conversely when the duodenal mesentery is abnormally short the space is reduced dorso-ventrally - 3 per cent. /

cent. (Nos. 56, 60, 61). This, or the presence of two hepatic glands - 8.0 per cent. (vide supra), or of an abnormally long gland - 1.0 per cent. (No. 66), will result in shortening of the trunk.

The Accessory Hepatic Lymph System

The Accessory Hepatic Group of Glands and Related Anomalies.- In 88.0 per cent. of these animals two glands, a cephalic and a caudal, were found. A single gland was present in 3.0 per cent. (Nos. 44, 66, 73), three glands in 6.0 per cent. (Nos, 9, 25, 26, 33, 36, 81) and four glands in 3.0 per cent. (Nos. 22, 27, 58).

Abnormalities of the Glands Themselves.- These were observed on five occasions.

(1) A large crescent-shaped cephalic gland associated with an underdeveloped caudal one was found in 2.0 per cent. (Nos. 20, 30).

(2) A large crescent-shaped caudal gland associated with a small cephalic gland was found in 1.0 per cent. (No. 24)

(3) As noted above a bridge of adenoid tissue connected the cephalic gland to the main hepatic gland itself in 2.0 per cent. (Nos. 31, 78).

Abnormalities /

Abnormalities of Drainage from These Glands.- In 94.0 per cent. of the animals examined all efferents from this group drained into the main hepatic system. On five occasions (Nos. 41, 42, 43, 60, 63) however, some efferents usually caudal ones joined the intestinal lymph trunk and once all of of them were seen to drain by this route (vide infra).

The Accessory Hepatic Lymph Trunk.- Efferent vessels from the accessory group of glands joined to form such a trunk usually in the region of the superior mesenteric vein in 12.0 per cent. (Nos. 3, 15, 18. 20, 22, 23, 30, 39, 41, 61, 78, 84). When present it was larger than the main hepatic lymph trunk itself and on six occasions it attained the normal size of that trunk (Nos. 3, 18, 20, 22, 23, 78). It is rarely seen to contain lymphatic valves. Normally it joins the main hepatic trunk but on one occasion it turned caudally over the superior mesenteric artery to join the intestinal trunk (No. 3). In one animal it was joined by the main hepatic lymph trunk ventral to the superior mesenteric vein (No. 91). Here the distal part of the common trunk thus formed contained two valves.

The /

The Effect of Such Abnormalities on the Feasibility of the Canulation Procedure

An attempt has been made arbitrarily to establish in what fraction of these animals there existed liver lymphatic trunks of a size suitable for canulation. This has been done on the basis of a fairly extensive experience with the technique.

Where no sizeable main or accessory trunk was present, where it was too short or where other anatomical abnormalities which prejudiced canulation, existed the arrangement has been regarded as 'unsuitable'. In a number of these preparations successful canulations were, in fact, obtained. When the dimensions of the lymphatic trunk were such as would have permitted an operator without special training in this kind of work to have performed a straightforward canulation the designation 'suitable' has been used.

The animals then fell into two groups:-

'Anatomically unsuitable for canulation' ..... 31.0 per cent. (Nos. 2, 6, 11, 15, 19, 30, 31, 32, 33, 36, 39, 41, 44, 45, 46, 54, 56, 60, 61, 66, 71, 75, 80, 81, 82, 83, 84, 85, 90, 95, 97).

'Anatomically suitable for canulation' ..... 69,0 per cent.

THE DISCUSSION

In a dynamic study of this type the increase in liver lymph flow which occurs during ether anaesthesia is of advantage. It helps to distend the lymphatic vessels and so to make them more clearly visible. No satisfactory explanation of this phenomenon is as yet available but it has been claimed that ether causes a reduction in plasma volume (McAllister, 1937; Stewart, unpublished work).

Distal ligation of the efferent collecting trunk near its site of entry into the cysterna produces marked retrograde engorgement. This is reflected in the distension of primary afferents on the portal vein and in the lesser omentum, all of which contributes to the ease and accuracy wherewith the whole hilar lymphatic system may be delineated. Conversely, it constitutes indirect evidence that most of the hilar lymph is being drained through that main vessel which has been occluded.

Not all of the lymph leaving the canine liver travels by the hilar route. It has been known for many years that some radicles follow the hepatic veins (Bollman, 1950; Morris, 1956). Under certain circumstances this pathway may carry more /

more than its share but normally it drains about 20.0 per cent. of all the lymph leaving the liver (Appendix B). Indeed, there is a well recognised reciprocity between these hilar and hepatic venous systems and this also may be observed between the main hepatic and accessory hepatic systems described above; so that, if one be blocked collaterals enlarge and shunt the lymph to patent pathways. Clearly therefore these two hilar systems are not entirely separate anatomical entities although, functionally, they seem to approximate to this. It is possible also that lymph vessels forming part of, or connections between, these two systems exist deeper in the tissues concerned and are not to be seen without extensive dissection.

It may be that in the large collecting vessels contamination of hepatic lymph with that from other tissues takes place. In particular the accessory hepatic group of glands may draw some lymph from the pancreas. Rarely a small intestinal lymph radicle flows into the main hepatic trunk. This is easily recognisable by its content of milky lymph and can be ligated if desired.

In 5.0 per cent. part, and in 1.0 per cent. all of the lymph from the accessory hepatic system joined the intestinal trunk. In the remaining 94.0 per cent. of these animals all the /

the hilar lymph seemed to be gathered into one common trunk before its discharge into the large abdominal collecting vessels. In this respect a comparison may be made with the lymphatic watershed from that area of the gut supplied by the superior mesenteric artery which in its turn finds its way into a common intestinal lymph trunk (Bollman et al., 1948). It was noted however that in 5.0 per cent. of the animals examined the hepatic and intestinal trunks finally joined to form a short common vessel which drained into the cysterna.

As might be expected there was considerable variation in the dimensions of the efferent trunks. Nonetheless a sizeable vessel was present in 69.0 per cent. of these preparations. In most cases this came from the main hepatic gland itself receiving near its origin efferents from the accessory group. In some however these accessory efferents themselves formed the major trunk and this trunk attained a size equal to that of the normal main hepatic trunk in 6.0 per cent. Thus, in about two-thirds of these animals it seemed that canulation of the lymphatic trunk draining most of the hilar lymph would have been a feasible and practical procedure.

The findings presented above concerning the number of vessels draining lymph from the main hepatic gland do not support /

support the current anatomical view that, while many afferents may drain into a lymphatic gland, only one efferent issues from its hilus (Gray, 1950). In 14.0 per cent. two, and in 3.0 per cent. three efferent trunks were seen to emerge from this gland. This can hardly be explained on the grounds of a regional or species difference. It is perhaps significant however that these trunks eventually did coalesce to form a short terminal vessel which entered the cysterna chyli.

SUMMARY

Abstracts of data obtained at operation during a study of the hilar lymphatics from 100 canine livers are presented.

Two afferent drainage pathways from the hilus are distinguished:-

(1) A 'Main Hepatic System' which drains lymph predominantly from the right lobes.

(2) An 'Accessory Hepatic System' which draws mainly on that from the left lobe.

Some abnormalities of the constituents of both systems are described and the frequency of their occurrence is given.

In /

In 94.0 per cent. of these preparations all of the hilar lymph seemed to pass into one common efferent trunk which then discharged it into the cysterna chyli.

69.0 per cent. were found to have a lymphatic vessel of such dimensions that canulation, for physiological studies, appeared to be both feasible and practical.

PROTOCOLS /

PROTOCOLS FOR APPENDIX A

Except where mentioned below the anatomy conformed to the normal pattern described in Appendix A. When the situation was such that no liver lymphatic trunk suitable for canulation presented, the phrase 'Anatomically Unsuitable' is used. The operations were performed consecutively and ether anaesthesia was used throughout.

Dog No. 1 Date of Op. 26.1.56. Successful Canulation

Two main hepatic glands were present. The main hepatic trunk was joined by the intestinal lymph trunk to form a common vessel just before it entered the cysterna chyli.

Dog No. 2. Date of Op. 30.1.56. Successful Canulation

The main hepatic trunk was poorly developed. The origin of the coeliac axis from the abdominal aorta was unusually low.

Anatomically unsuitable.

Dog No. 3. Date of Op. 2.2.56. Successful Canulation

The /

Dog No. 3. Date of Op. 2.2.56. Successful Canulation

The main hepatic trunk was underdeveloped. A large accessory hepatic trunk was found. It turned caudally over the superior mesenteric artery to join the intestinal trunk. This trunk was canulated.

Dog No. 4. Date of Op. 8.2.56. Unsuccessful Canulation

An atopic gland was present in the right free edge of the lesser omentum cephalad to the coeliac artery and dorsal to the portal vein. The hepatic gland itself was mobile and suspended in a peritoneal fold which arose from the usual site so that the gland lay superficial to the duodenal mesentery. The origin of the coeliac artery was low but the main hepatic trunk itself was of normal dimensions. Technically canulation should have been possible.

Dog No. 5. Date of Op. 14.2.56. Successful Canulation

Two hepatic glands were present at the usual site.

Dog No. 6. Date of Op. 16.2.56. Unsuccessful Canulation

The main hepatic trunk was small. The origin of the superior mesenteric artery from the abdominal aorta was abnormally high. There was an excess of fat in the duodenal mesentery.

Anatomically unsuitable.

Dog. No. 7. /

Dog. No. 7. Date of Op. 20.2.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 8. Date of Op. 27.2.56. Successful Canulation

The origin of the superior mesenteric artery was abnormally high. The main hepatic trunk was well developed. There was an excess of fat in the duodenal mesentery.

Dog No. 9. Date of Op. 1.3.56. Successful Canulation

The main hepatic trunk was joined by the intestinal lymph trunk to form a common vessel just before it entered the cysterna chyli. Three accessory hepatic glands were present.

Dog No. 10. Date of Op. 5.3.56. Unsuccessful Canulation

The main hepatic trunk was joined by the intestinal lymph trunk to form a common vessel just before it entered the cysterna chyli. Technically it should have been possible to canulate the main hepatic trunk above this level.

Dog No. 11. Date of Op. 8.3.56. Unsuccessful Canulation

There were only two afferent radicles in the main hepatic system on the portal vein. A large group entered the accessory hepatic system from the lesser omentum. The main hepatic trunk was small.

Anatomically unsuitable.

Dog No. 12 /

Dog No. 12. Date of Op. 9.3.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 13. Date of Op. 12.3.56. Successful Canulation

Two main hepatic glands were present at the usual site.

Dog No. 14. Date of Op. 15.3.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 15. Date of Op. 19.3.56. Unsuccessful Canulation

Two main hepatic glands were present at the usual site. The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high. Most of the afferents seemed to be going to the accessory hepatic group of glands. An accessory hepatic lymph trunk was present but this was also small.

Anatomically unsuitable.

Dog No. 16. Date of Op. 21.3.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava. The origin of the superior mesenteric artery was abnormally high.

Dog No. 17. /

Dog No. 17. Date of Op. 26.3.56. Unsuccessful Canulation

The main hepatic trunk had undergone cavernous transformation. The origin of the superior mesenteric artery was abnormally high. Technically canulation of the cavernous trunk should have been possible.

Dog No. 18. Date of Op. 28.3.56. Successful Canulation

The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high. A large accessory hepatic trunk was found and this was canulated.

Dog No. 19. Date of Op. 2.4.56. Unsuccessful Canulation

Two main hepatic glands were present at the usual site. The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high and that of the coeliac axis abnormally low.

Anatomically unsuitable.

Dog No. 20. Date of Op. 5.4.56. Successful Canulation

The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high and that of the coeliac axis abnormally low. Most of the afferents from the hilus drained into the accessory hepatic group of glands which /

which was represented by a large crescent-shaped cephalic gland and an underdeveloped caudal one. An accessory hepatic trunk was present and suitable in size. This trunk was canulated.

Dog No. 21. Date of Op. 9.4.56. Successful Canulation

The main hepatic trunk was joined by the intestinal lymph trunk to form a common vessel just before it entered the cysterna chyli.

Dog No. 22. Date of Op. 12.4.56. Successful Canulation

The main hepatic trunk was small. Four glands were present in the accessory hepatic group. A large accessory hepatic trunk was found and this was canulated.

Dog No. 23. Date of Op. 13.4.56. Successful Canulation

The main hepatic trunk was small and bifid. A large accessory hepatic trunk was present and this was canulated.

Dog No. 24. Date of Op. 16.4.56. Successful Canulation

The main hepatic trunk was large and exhibited cavernous transformation. The accessory hepatic group was made up of a large crescent-shaped caudal gland associated with a small cephalic gland.

Dog No. 25. /

Dog No. 25. Date of Op. 18.4.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava. The origin of the superior mesenteric artery was abnormally high. Three glands were present in the accessory hepatic group.

Dog No. 26. Date of Op. 23.4.56. Successful Canulation

Three glands were present in the accessory hepatic group.

Dog No. 27. Date of Op. 25.4.56. Successful Canulation

Four glands were present in the accessory hepatic group.

Dog No. 28. Date of Op. 30.4.56. Successful Canulation

The main hepatic trunk was represented by three discrete trunks; the most ventral of which was canulated.

Dog No. 29. Date of Op. 2.5.56. Successful Canulation

The main hepatic trunk was small but was dilated almost to normal size in its proximal part. The origin of the superior mesenteric artery was abnormally high.

Dog No. 30. /

Dog No. 30. Date of Op. 7.5.56. Successful Canulation

The main hepatic trunk was small. The accessory hepatic group was made up of a large crescent-shaped cephalic gland associated with a small caudal gland. Most of the afferents seemed to be entering this group. An accessory hepatic trunk was present and this was canulated with some difficulty.

Anatomically unsuitable.

Dog No. 31. Date of Op. 9.5.56. Unsuccessful Canulation

The main hepatic gland was connected by a bridge of adenoid tissue passing ventrally over the right side of the superior mesenteric vein to the cephalic gland in the accessory hepatic group. The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 32. Date of Op. 11.5.56. Successful Canulation

The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high. A large efferent came from the cephalic gland in the accessory hepatic group and this was canulated.

Anatomically unsuitable.

Dog No. 33. /

Dog No. 33. Date of Op. 14.5.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava. Three glands were present in the accessory hepatic group.

Anatomically unsuitable.

Dog No. 34. Date of Op. 16.5.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 35. Date of Op. 22.5.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava.

Dog No. 36. Date of Op. 23.5.56. Unsuccessful Canulation

The main hepatic trunk was represented by a short stump. The origin of the superior mesenteric artery was abnormally high. Three glands were present in the accessory hepatic group.

Anatomically unsuitable.

Dog No. 37. /

Dog No. 37. Date of Op. 25.5.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 38. Date of Op. 28.5.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 39. Date of Op. 30.5.56. Successful Canulation

No main hepatic trunk was seen. The origin of the superior mesenteric artery was abnormally high. An accessory hepatic trunk was present and this was canulated with difficulty.

Anatomically unsuitable.

Dog No. 40. Date of Op. 1.6.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 41. Date of Op. 4.6.56. Successful Canulation

The main hepatic trunk was small but exhibited a slight bulbous dilatation near its origin. It was canulated at this site with some difficulty. The origin of the superior mesenteric artery was abnormally high. Efferents from glands in the accessory hepatic group joined the intestinal lymph trunk after forming an accessory hepatic trunk.

Anatomically unsuitable.

Dog No. 42. /

Dog No. 42. Date of Op. 6.6.56. Successful Canulation

The origin of the superior mesenteric artery was abnormally high. Efferents from the accessory hepatic group joined the intestinal lymph trunk.

Dog No. 43. Date of Op. 8.6.56. Successful Canulation

The main hepatic trunk was small but exhibited a slight bulbous dilatation near its origin. It was canulated at this site. The origin of the superior mesenteric artery was abnormally high. One efferent from the accessory hepatic group joined the intestinal lymph trunk.

Dog No. 44. Date of Op. 11.6.56. Successful Canulation

The main hepatic trunk was bifid for most of its length and the dorsal branch was canulated. Only one accessory hepatic gland was present.

Anatomically unsuitable.

Dog No. 45. Date of Op. 13.6.56. Successful Canulation

The main hepatic trunk was abnormally short and exhibited cavernous transformation. Canulation was only possible by first passing the catheter under the inferior vena cava. The origin of the superior mesenteric artery was abnormally high and that of the coeliac axis abnormally low.

Anatomically unsuitable.

Dog No. 46. /

Dog No. 46. Date of Op. 18.6.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava.

Anatomically unsuitable.

Dog No. 47. Date of Op. 20.6.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 48. Date of Op. 22.6.56. Successful Canulation

The course of the main hepatic trunk was abnormal. It crossed the superior mesenteric artery and the left renal vein before turning dorsally into the cysterna chyli.

Dog No. 49. Date of Op. 25.6.56. Successful Canulation

The main hepatic trunk was smaller than usual.

Dog No. 50. Date of Op. 27.6.56. Unsuccessful Canulation

No anatomical abnormality was noted. The tip of the catheter was advanced to the region of a valve which lay at the point where the main hepatic trunk was joined by the efferents from the accessory hepatic group. A good flow of lymph was obtained but this failed after the wound was closed. This was probably a technical fault in that the catheter was not passed through the valve.

Dog No. 51. /

Dog No. 51. Date of Op. 29.6.56. Successful Canulation

The main hepatic trunk was smaller than usual. The origin of the superior mesenteric artery was abnormally high.

Dog No. 52. Date of Op. 3.7.56. Successful Canulation

The origin of the superior mesenteric artery was abnormally high. Two nodes of accessory pancreatic tissue lay between the hepatic gland and the superior mesenteric vein.

Dog No. 53. Date of Op. 5.7.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 54. Date of Op. 6.7.56. Successful Canulation

The main hepatic trunk was small.

Anatomically unsuitable.

Dog No. 55. Date of Op. 9.7.56. Successful Canulation

There was an excess of fat in the duodenal mesentery.

Dog No. 56. Date of Op. 10.7.56. Successful Canulation

Some afferents in the main hepatic system swung dorsally over the coeliac artery before turning forwards to the hepatic gland. The main hepatic trunk was bifid and abnormally short. The ventral division was canulated with difficulty and to do this /

this the catheter had first to be passed under the inferior vena cava. The duodenal mesentery was abnormally short. The left renal vein joined the inferior vena cava at an abnormally high level.

Anatomically unsuitable.

Dog No. 57. Date of Op. 16.7.56. Successful Canulation

Two main hepatic glands were present at the usual site.

Dog No. 58. Date of Op. 18.7.56. Successful Canulation

Two main hepatic glands were present at the usual site. Four glands were present in the accessory hepatic group. The main hepatic trunk was torn during an attempt at canulation and the catheter had to be reinserted at a higher site than usual.

Dog No. 59. Date of Op. 19.7.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 60. Date of Op. 23.7.56. Unsuccessful Canulation

The main hepatic gland was mobile and suspended in a peritoneal fold. It lay deep in the foramen of Winslow behind the coeliac axis and could not be seen until displaced outwards. The main hepatic trunk was very small and bifid.

The /

The origin of the superior mesenteric artery was abnormally high and that of the coeliac axis abnormally low. The duodenal mesentery was unduly short. One efferent from the accessory hepatic group joined the intestinal lymph trunk.

Anatomically unsuitable.

Dog No. 61. Date of Op. 24.7.56. Successful Canulation

The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high. The duodenal mesentery was unduly short. An accessory hepatic trunk was present and this was canulated with difficulty.

Anatomically unsuitable.

Dog No. 62. Date of Op. 26.7.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 63. Date of Op. 27.7.56. Successful Canulation

The main hepatic trunk exhibited cavernous transformation. One efferent from the accessory group joined the intestinal lymph trunk.

Dog No. 64. Date of Op. 30.7.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 65. /

Dog No. 65. Date of Op. 31.7.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 66. Date of Op. 1.8.56. Unsuccessful Canulation

The main hepatic gland was found to be long, thin and tapered reaching backwards almost to the inferior vena cava, consequently it gave off a very short main hepatic trunk. The origin of the superior mesenteric artery was abnormally high. Only one accessory hepatic gland was present. There was an excess of fat in the duodenal mesentery.

Anatomically unsuitable.

Dog No. 67. Date of Op. 2.8.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 68. Date of Op. 2.8.56. Unsuccessful Canulation

No anatomical abnormality was noted. The main hepatic trunk was thin-walled and the point of the catheter had not been adequately smoothed off so that it pierced the vessel wall. This was a technical error.

Dog No. 69. Date of Op. 6.8.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 70. /

Dog No. 70. Date of Op. 7.8.56. Successful Canulation

The proximal part of the main hepatic trunk was bifid but the ventral division was quite large and was canulated without difficulty. It crossed the superior mesenteric artery before turning dorsally to join the cysterna chyli.

Dog No. 71. Date of Op. 9.8.56. Successful Canulation

The main hepatic trunk was represented by three discrete vessels of which the middle vessel was canulated with difficulty.

Anatomically unsuitable.

Dog No. 72. Date of Op. 10.8.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 73. Date of Op. 13.8.56. Successful Canulation

Only one accessory hepatic gland was present.

Dog No. 74. Date of Op. 14.8.56. Successful Canulation

No anatomical abnormality was noted. The main hepatic trunk was thin-walled so that it was punctured by the tip of the catheter. This happened three times and eventually at the fourth attempt the catheter was inserted in the most proximal part of the trunk.

Dog No. 75. /

Dog No. 75. Date of Op. 16.8.56. Unsuccessful Canulation

The main hepatic trunk was represented by three small discrete vessels. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 76. Date of Op. 17.8.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 77. Date of Op. 20.8.56. Successful Canulation

The main hepatic trunk exhibited cavernous transformation. The origin of the superior mesenteric artery was abnormally high.

Dog No. 78. Date of Op. 23.8.56. Successful Canulation

The main hepatic gland was connected by a bridge of adenoid tissue passing ventrally over the right side of the superior mesenteric vein to the cephalic gland in the accessory hepatic group. The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high. A large accessory hepatic trunk was present and this was canulated.

Dog No. 79./

Dog No. 79. Date of Op. 24.8.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 80. Date of Op. 27.8.56. No canulation attempted

The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 81. Date of Op. 28.8.56. Unsuccessful Canulation

The main hepatic trunk was bifid. It crossed the superior mesenteric artery and the left renal vein before turning dorsally into the cysterna chyli. Three glands were present in the accessory hepatic group.

Anatomically unsuitable.

Dog No. 82. Date of Op. 30.8.56. Unsuccessful Canulation

An atopic lymph gland draining into the main hepatic system was found cephalad to the splenic vein and medial to the portal vein. The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 83. /

Dog No. 83. Date of Op. 31.8.56. Successful Canulation

The main hepatic trunk was rather small and abnormally short. Canulation was only possible by first passing the catheter under the inferior vena cava to reach the distal part of the trunk which crossed the superior mesenteric artery and the left renal vein before turning dorsally into the cysterna chyli.

Anatomically unsuitable.

Dog No. 84. Date of Op. 4.9.56. Successful Canulation

The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high. An accessory hepatic trunk was present and this was canulated with difficulty.

Anatomically unsuitable.

Dog No. 85. Date of Op. 5.9.56. Successful Canulation

Two main hepatic glands were present at the usual site. The main hepatic trunk was small and bifid and the posterior division was canulated with difficulty. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 86. /

Dog No. 86. Date of Op. 6.9.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 87. Date of Op. 10.9.56. Successful Canulation

The main hepatic gland was firmly adherent to the superior mesenteric vein. The main hepatic trunk was bifid in its proximal half so that the definitive trunk was abnormally short. Canulation was only possible by first passing the catheter under the inferior vena cava.

Dog No. 88. Date of Op. 11.9.56. No Canulation Attempted

No anatomical abnormality was noted.

Dog No. 89. Date of Op. 11.9.56. No Canulation Attempted

No anatomical abnormality was noted.

Dog No. 90. Date of Op. 11.9.56. No Canulation Attempted

The main hepatic trunk was small and bifid. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 91. Date of Op. 12.9.56. Successful Canulation

The main hepatic trunk turned ventrally over the right side of the superior mesenteric vein to join an accessory hepatic trunk. The common trunk thus formed turned sharply backwards /

backwards to empty into the cysterna chyli at the usual site. It was seen to contain two valves and was easily canulated. A node of accessory pancreatic tissue was found at the site usually occupied by the main hepatic gland. As a result of this the gland was displaced caudally.

Dog No. 92. Date of Op. 13.9.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 93. Date of Op. 13.9.56. Successful Canulation

No anatomical abnormality was noted.

Dog No. 94. Date of Op. 14.9.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava.

Dog No. 95. Date of Op. 14.9.56. No Canulation Attempted

The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 96. /

Dog No. 96. Date of Op. 18.9.56. Successful Canulation

The main hepatic trunk was abnormally short. Canulation was only possible by first passing the catheter under the inferior vena cava.

Dog No. 97. Date of Op. 19.9.56. No Canulation Attempted

The main hepatic trunk was small. The origin of the superior mesenteric artery was abnormally high.

Anatomically unsuitable.

Dog No. 98. Date of Op. 20.9.56. No Canulation Attempted

No anatomical abnormality was noted.

Dog No. 99. Date of Op. 20.9.56. Successful Canulation

The main hepatic trunk was abnormally short and canulation was only possible by first passing the catheter under the inferior vena cava. It joined the intestinal lymph trunk to form a common vessel just before it entered the cysterna chyli.

Dog No. 100. Date of Op. 21.9.56. No Canulation Attempted

No anatomical abnormality was noted.

APPENDIX B

STUDIES ON
THE FLOW OF LYMPH
FROM THE CANINE LIVER

STUDIES ON THE FLOW OF LYMPH FROM THE CANINE LIVER

I.

SIGNIFICANCE OF FLOW THROUGH THE HEPATIC VENOUS LYMPH SYSTEM

This study was undertaken to ascertain what fraction of the lymph leaving the canine liver travels by those lymph vessels which follow the hepatic veins (Appendix A) or, conversely, how much of the total liver lymph flow was being collected in the type of preparation used in this thesis in which only the hilar lymph system is canulated. To do this use was made of the fact that bilirubin passes freely into the lymph during the first few hours of biliary obstruction in the cholecystectomised animal (vide supra).

In five animals (Nos. 1 to 5) canulas were inserted into both the liver lymphatic trunk which drains the hilar lymph and the thoracic duct into which flows the lymph from the hepatic venous lymph system (Appendix A). The gall bladder was removed and the common bile duct was doubly tied and severed. A third pathway of drainage from the liver is described in man (Cunningham, 1951), namely via plexi on the abdominal /

abdominal and thoracic surfaces of the diaphragm to the internal mammary and mediastinal lymph trunks. There is no counterpart to this in the canine anatomy (Appendix A.). It is assumed that the concentration of bilirubin in the liver lymph generally and, since there is free interconnection between them in lymph from the hilar and hepatic venous systems specifically(Appendix A), is uniform at any one time. It was possible to make a quantitative calculation of the output of bilirubin through the usual hilar catheter during a fixed period of obstruction by measuring the volume of lymph collected and the mean concentration of bilirubin therein. By the same method it was possible to estimate the output of bilirubin into the thoracic duct lymph during this period. By adding these totals the total output of bilirubin from the liver, during this time, could be found. The relative percentages of the total output passing via the hilar and via the hepatic venous systems could now be calculated. Since the concentrationsof bilirubin in the lymph leaving the liver by both routes should be similar the volume flowing through either route will be directly proportional to the output of pigment from each.

It is possible that biliary obstruction may alter the flow ratios between these two routes but there does not seem to /

to be any reason for assuming that this does in fact take place.

Results

These are given in detail in the protocols at the end of this appendix. A synopsis only is presented at this stage.

Experiment No.	% Bilirubin in Hilar Lymph	% Bilirubin in Hep. Ven. Lymph	Duration of Study in hr.
I.	83	17	5
II.	95	5	5
III.	70	30	5
IV.	89	11	5
V.	78	22	6½
	415	85	

	Mean Output	Range
Hilar Lymph	83%	70-95%
Hep. Ven. Lymph	17%	5-30%

Discussion

No studies were found in the literature concerning the relative percentages of the total lymph leaving the canine liver /

liver which travel by the hilar and hepatic venous routes. This has also been pointed out by Morris (1956). Bollman (1950) has stated that he believes the hep. venous fraction to be a small one.

Only five experiments are presented in this study and hence the statistical significance of the results is questionable. Nevertheless it seems that in these animals at least, about 80 per cent. of all the lymph-bourne bilirubin leaving the liver during the regurgitation phase went by the hilar route. If these findings are correct it follows that about 20 per cent. of the total liver lymph flow passed through the hepatic venous lymph system into the thoracic duct.

A scrutiny of the protocols (1 to 5) will show that, although the liver lymph was being withdrawn through both hilar and thoracic duct fistulae the concentration of direct-reacting bilirubin in the venous blood of these animals more than doubled itself during the five or six hour period of study. This suggests that direct-reacting bilirubin was also passing into the blood traversing the liver at this stage (Bollman et al., 1927). The actual rise in concentration seems small but this is due, in part at least, to the fact that it is at once diluted by the total blood volume /

volume whereas such bilirubin as passes into the liver lymph is diluted by a much smaller volume of fluid.

Summary

The gall bladder was removed and the common bile duct was ligated in five canine preparations having both liver lymphatic and thoracic duct fistulae.

Measurements were made of the relative percentages of the total amount of bilirubin leaving the liver during the next five or six hours which passed by each route.

It was found that about 80 per cent. of all the bilirubin recovered from both catheters left by the hilar route.

It is argued that about 80 per cent. of the lymph leaving the canine liver probably travels by the hilar route and that hence the hepatic venous lymph system carries about 20 per cent.

STUDIES /

STUDIES ON THE FLOW OF LYMPH FROM THE CANINE LIVER

II.

FLOW OF LYMPH FROM THE CANINE LIVER DURING BILIARY OBSTRUCTION

This study was undertaken for two reasons. Firstly, it was necessary to estimate the fluid loss suffered by the unanaesthetised animal with a liver lymphatic fistula during biliary obstruction, so that some guidance could be obtained as to fluid replacement. Secondly, no data were found in the literature relating to lymph flow from the canine liver under these circumstances.

Cain (1947) measured liver lymph flow in sixteen unanaesthetised dogs from which the gall bladder had been removed and found this to be 10.98 ml. per hour. In ten such dogs under ether anaesthesia it was 17.76 ml. per hour. The weight of these animals was not considered in the calculation but he stated that they were about 15 kg. He measured the flow in three anaesthetised animals after ligation of the common bile duct. The periods of measurement were $1\frac{1}{2}$, 1, and $\frac{3}{4}$ hours respectively. He consideredthat these studies were inconclusive but noted that no significant increase occurred.

Morris /

Morris (1956) found liver lymph flow to be 0.75 ± 0.06 ml. per kg. per hr. in 38 normal cats under nembutal anaesthesia. In the normal rat (200 g.) Bollman et al. (1948) found it to be 5 ml. per day. While Friedman et al. (1956) reported a flow increase of five times over normal in the twelve hours following biliary obstruction in six rats. Their figures for normal flow are unusually low (Bollman, Personal Communication). No account was taken of hepatic venous lymph flow in the above studies.

Accordingly, throughout periods of biliary obstruction lasting for up to seventy-two hours the flow of hilar lymph from the liver was measured in 20 unanaesthetised dogs (A to T.). The animals weighed between 10 and 12 kg. In 11 of these preparations (A, C, D, E, G, H, N, O, R, S, T), cholecystectomy had been carried out at the time of insertion of the lymphatic catheter. From four animals (J, M, P, Q.) the gall bladder had been removed three to four weeks previously and in four (F, I, K, L.) it was left in situ. In the remaining preparations (B) biliary obstruction had been present for five weeks before the lymphatic catheter was inserted.

Results /

Results

These are given in detail in the protocols at the end of this appendix.

The mean flow of hilar lymph from the fistulae in these 20 animals (A to T) was found to be 0.66 ml. per kg. per hr. with a standard error of ± 0.07.

In a 10 kg. animal this would represent a fluid loss of 158.4 ml. per day.

Discussion

These findings are similar to those reported by Cain (1947) in his studies on normal animals. It seems that the weight of his animals was about 15 kg. and if this was so his figure of 10.98 ml. per hr. may be compared with 9.9 ml. per hr., which would be forthcoming from the data presented in this study in a hypothetical 15 kg. animal. This would suggest that the presence of biliary obstruction has no lasting effect on the flow of lymph from the canine liver, but such a subject is not related to the main argument of this thesis.

A scrutiny of the protocols at the end of this appendix will show that there was a considerable variation in the values /

values obtained. Apart from experiment B, where the obstruction was chronic, it will be seen that high flows were confined to the recently cholecystectomised group. The increase was most marked when the flow was measured soon after operation and this seems to confirm the finding of Cain (1947) that ether anaesthesia augments the flow of lymph from the canine liver.

In the previously cholecystectomised animals and in those from which the gall bladder had not been removed flows were consistently below the mean. The number of experiments in these sub-groups, however, was small and no further analysis of the data would be purposeful.

If the findings described in the previous study are correct this figure of 0.66 ml. per kg. per hr. probably represents about 80 per cent. of the total volume of lymph leaving the liver during biliary obstruction.

Summary /

Summary

The flow of hilar lymph from the canine liver has been measured in 20 unanaesthetised animals during biliary obstruction.

The mean flow was found to be 0.66 ml. per kg. per hr. The standard error of the mean was ± 0.07.

In a 10 kg. animal this would represent a flow of about 150 ml. per day.

PROTOCOLS FOR APPENDIX B: PAPER I.

BILIRUBIN STUDIES

*T.D. & L.L.
Experiment 1.
Dog 1.
24.8.56.

G.B. out
C.B.D. Lig. & Div.
T.D. L.L.

Ducts tied 9.45 a.m. (After canulation)
Op. finished 10 a.m.

Time	Hilar Lymph Conc. mg.%	Hilar Lymph Vol. ml.	Hilar Lymph Total output mg.	Thoracic Duct Lymph Conc. mg.%	Thoracic Duct Lymph Vol. ml.	Thoracic Duct Lymph Total output mg.	Blood Bil. mg.%
10 a.m	2.22	9.0	0.2358	0.04	36.0	0.0252	0.07
11	2.62			0.07			0.18
11-12	6.10 7.0	7.0	0.4900	0.1 0.12	27.0	0.0324	
12-1 p.m.	9.40 10.62	9.0	0.9558	1.0 1.3	15.0	0.195	
1-2	10.56 11.87	11.5	1.3650	1.4 1.62	18.0	0.2916	
							3 p.m.
2-3	11.71 13.30	10.5	1.3965	1.4 1.62	20.0	0.3240	0.26 0.48
			4.4431			0.8682	
			0.8682				
			5.3113				

Total output in hilar lymph 83%

Total output in thoracic duct lymph 17%

(*T.D. and L.L. = Thoracic duct and liver lymph fistulae)

BILIRUBIN STUDIES

T.D. & L.L.
Experiment 2
Dog 2.
20.8.56.

G.B. out
C.B.D. Lig. & Div.
T.D. L.L.

Ducts tied 10 a.m. (After canulation)
Op. finished 10.30 a.m.

Time	Hilar Lymph Conc. mg.%	Hilar Lymph Vol. ml.	Hilar Lymph Total output mg.	Thoracic Duct Lymph Conc. mg.%	Thoracic Duct Lymph Vol. ml.	Thoracic Duct Lymph Total output mg.	Blood Bil. mg.%
10.45-11.45	1.81 2.25	8.0	0.2	0.33 0.50	11.5	0.05	0.087 0.087
11.45-12.45	8.58 10.0	8.0	0.8	0.13 0.2	10.5	0.02	-
12.45-1.45	15.34 16.0	10.5	1.68	0.49 0.5	10.2	0.05	-
1.45-2.45	15.65 16.75	15.0	2.51	0.45 0.50	13.0	0.06	- 3.45 p.m.
2.45-3.45	14.85 15.75	20.0	3.15	0.99 1.7	15.5	0.22	0.21 0.32
			8.34 0.40 8.74			0.40	

Total output in hilar lymph 95%

Total output in thoracic duct lymph 5%

BILIRUBIN STUDIES

T.D. & L.L.
Experiment 3.
Dog 3.
17.8.56.

G.B. out
C.B.D. Lig. & Div.
T.D. L.L.

Ducts tied 10 a.m. (After canulation)
Op. finished 10.30 a.m.

Time	Hilar Lymph		Total	Thoracic Duct Lymph		Total	Blood
	Conc. mg.%	Vol. ml.	output mg.	Conc. mg.%	Vol. ml.	output mg.	Bil. mg.%
10.30-	2.39	6.0	0.165	0.39	9.3	0.041	0.24
11.30	2.75			0.45			0.30
11.30-	5.19	8.0	0.512	0.67	11.3	0.098	-
12.30	6.40			0.87			
12.30-	9.07	5.2	0.507	1.89	8.3	0.195	-
1.30	9.70			2.35			
1.30-	10.36	4.3	0.466	3.06	7.6	0.245	-
2.30	10.84			3.25			3.30
2.30-	11.55	4.8	0.587	4.78	7.5	0.375	p.m.
3.30	12.25			5.0			0.51
							0.67
			2.237			0.954	
			0.954				
			3.191				

Total output in hilar lymph 70%

Total output in thoracic duct lymph 30%

BILIRUBIN STUDIES

T.D. & L.L.
Experiment 4.
Dog 4.
20.9.56.

G.B. out
C.B.D. Lig. & Div.
T.D. & L.L.

Ducts tied at 9.45 a.m. (After canulation)
Op. finished 10 a.m.

Time	Hilar Lymph Conc. mg.%	Hilar Lymph Vol. ml.	Total output mg.	Thoracic Duct Lymph Conc. mg.%	Thoracic Duct Lymph Vol. ml.	Total output mg.	Blood Bil. mg.%
10a.m.	8.05	50.0	4.125	1.48	35.0	0.5	0.06
3 p.m.	8.25			1.75			0.098
			4.125			0.5	3 p.m.
			0.5				0.24
			4.625				0.38

Total output in hilar lymph 89%

Total output in thoracic duct lymph 11%

BILIRUBIN STUDIES

T.D. & L.L.
Experiment 5.
Dog 5.
18.9.56.

G.B. out
C.B.D. Lig. & Div.
T.D. & L.L.

Ducts tied at 9.45 a.m. (After canulation)
Op. finished 10 a.m.

Time	Hilar Lymph Conc. mg.%	Hilar Lymph Vol. ml.	Total output mg.	Thoracic Duct Lymph Conc. mg.%	Thoracic Duct Lymph Vol. ml.	Total output mg.	Blood Bil. mg.%
10 am	10.23	115	12.9375	3.06	115	3.565	0.08
4.30 p.m.	11.25			3.10			0.20
			12.9375			3.565	
			3.565				
			16.5025				4.30 p.m.
							0.52
							0.63

Total output in hilar lymph 78%

Total output in thoracic duct lymph 22%

PROTOCOLS FOR APPENDIX B: PAPER II.

	Duration of Measurements	Lymph Flow
Dog A. Wt. 10 kg. 7.8.56.	Obstruct C.B.D. tube 3 hr. post-op.	G.B. out rec. C.B.D. tube Liver lymph
	24 hr.	116.0 ml.
Lymph flow was 0.48 ml. per kg. per hr.		
Dog B. Wt. 12 kg. 2.3.56.	Five week obstruction.	G.B. out chron. Lig. & Div. C.B.D. Liver lymph
	5 hr.	69.4 ml.
Lymph flow was 1.2 ml. per kg. per hr.		
Dog C. Wt. 12 kg. 2.3.56.	Obstruct C.B.D. tube 25 hr. post-op.	G.B. out rec. C.B.D. tube Liver lymph
	5 hr.	37.0 ml.
Lymph flow was 0.62 ml. per kg. per hr.		
Dog D. Wt. 10 kg. 25.4.56.	Obstructed at 24 hr. post-op.	G.B. out rec. Snare C.B.D. Liver lymph
	4 hr.	33.2 ml.
Lymph flow was 0.83 ml. per kg. per hr.		
Dog E. Wt. 12 kg. 21.3.56.	Obstruct C.B.D. tube 2½ hr. post-op.	G.B. out rec. C.B.D. tube Liver lymph
	3 hr.	45.2 ml.
Lymph flow was 1.3 ml. per kg. per hr.		

Dog. F. /

	Duration of Measurements	Lymph Flow
Dog F. Wt. 12 kg. 22.5.56.	Obstructed at 5 hr. post-op.	G.B. in Snare C.B.D. Liver lymph
	17 hr.	90.5 ml.
Lymph flow was 0.44 ml. per kg. per hr.		
Dog G. Wt. 12 kg. 30.8.56.	Obstruct C.B.D. tube 2 hr. post-op.	G.B. out rec. C.B.D. tube Liver lymph
	24 hr.	199.0 ml.
Lymph flow was 0.69 ml. per kg. per hr.		
Dog H. Wt. 10 kg. 15.3.56.	Obstruct C.B.D. tube 3 hr. post-op.	G.B. out rec. C.B.D. tube Liver lymph
	2 hr.	17.9 ml.
Lymph flow was 0.89 ml. per kg. per hr.		
Dog I. Wt. 11 kg. 13.6.56.	Obstructed at operation	G.B. in Lig. & Div. C.B.D. Liver lymph
	72 hr.	380 ml.
Lymph flow was 0.48 ml. per kg. per hr.		
Dog J. Wt. 12 kg. 11.6.56.	Obstructed at operation	G.B. out prev. Lig. & Div. C.B.D. Liver lymph
	36 hr.	228 ml.
Lymph flow was 0.52 ml. per kg. per hr.		

Dog K. /

	Duration of Measurements	Lymph flow
Dog K. Wt. 10 kg. 28.5.56.	Obstructed at operation	G.B. in Lig. & Div. C.B.D. Liver lymph
	36 hr.	122.0 ml.

Lymph flow was 0.34 ml. per kg. per hr.

Dog L. Wt. 12 kg. 25.5.56.	Obstructed at operation	G.B. in Lig. & Div. C.B.D. Liver lymph
	24 hr.	130.0 ml.

Lymph flow was 0.45 ml. per kg. per hr.

Dog M. Wt. 11 kg. 6.6.56.	Obstructed at 4 hr. post-op.	G.B. out prev. Snare C.B.D. Liver lymph
	24 hr.	65.0 ml.

Lymph flow was 0.24 ml. per kg. per hr.

Dog N. Wt. 12 kg. 28.2.56.	Obstruct C.B.D. tube 30 hr. post.-op.	G.B. out rec. C.B.D. tube Liver lymph
	4 hr.	25.1 ml.

Lymph flow was 0.52 ml. per kg. per hr.

Dog O. Wt. 10 kg. 25.6.56.	Obstructed at operation	G.B. out rec. Inject vinyl ac. C.B.D. Liver lymph
	24 hr.	246.0 ml.

Lymph flow was 1.0 ml. per kg. per hr.

Dog P. /

	Duration of Measurements	Lymph Flow
Dog P. Wt. 11 kg. 9.7.56.	Obstructed at operation	G.B. out prev. Lig. & Div. C.B.D. Liver lymph
	24 hr.	95.0 ml.

Lymph flow was 0.35 ml. per kg. per hr.

Dog Q. Wt. 10 kg. 10.7.56.	Obstructed at operation	G.B. out prev. Lig. & Div. C.B.D. Liver lymph
	24 hr.	120.0 ml.

Lymph flow was 0.50 ml. per kg. per hr.

Dog R. Wt. 11 kg. 16.7.56.	Obstructed at operation	G.B. out rec. Lig. & Div. C.B.D. Liver lymph
	72 hr.	548.0 ml.

Lymph flow was 0.69 ml. per kg. per hr.

Dog S. Wt. 10 kg. 6.8.56.	Obstruct C.B.D. tube 3 hr. post.-op.	G.B. out rec. C.B.D. tube Liver lymph
	3 hr.	34.0 ml.

Lymph flow was 1.1 ml. per kg. per hr.

Dog T. Wt. 10 kg. 31.7.56.	Obstructed at operation	G.B. out rec. Lig. & Div. C.B.D. Liver lymph
	24 hr.	150.0 ml.

Lymph flow was 0.62 ml. per kg. per hr.
