THE EARLY PHASE OF EXPERIMENTAL EXTRAHEPATIC BILIARY OBSTRUCTION

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It has long been known that complete biliary obstruction is one of the causes of jaundice, a condition in which an excess of bile pigment is found in the plasma and passed on to the tissues which in time become pale-yellow in colour. The mechanism involved in the production of obstructive jaundice is, however, far from clear. Experimental ligation of the common hepatic duct in dogs produces a detectable rise in the concentration of bilirubin in the plasma within a day or two, but when the cystic duct also is ligated, or the gallbladder rendered nonfunctional by some other means, the rise in the plasma concentration of bilirubin may be detected in from 5 minutes to 3 hours depending on the sensitivity of the test applied. It is generally accepted that this hyperbilirubinaemia may be a result of regurgitation, that is, a reflux to the vascular system of substances previously secreted into the bile channels. This is, however, a concept which requires that the secretory mechanism continues to function during obstruction, which it may not do in all circumstances. Thus, while the secretory mechanism appears to be undamaged by short periods of experimental biliary obstruction, inasmuch as the renewed rate of flow and the composition of the bile is normal, this is not the case where the period of obstruction is prolonged. For some time following release after prolonged obstruction the bile flow is found to be very slow or absent and the liver is unable to concentrate, or even excrete, test substances. Again, while hepatic lymph collected in the early stage of a period of biliary obstruction is found to contain remarkably high concentrations of bilirubin, concentrations greatly in excess of those found in the plasma, this picture changes with continued obstruction and in time the bilirubin concentration in the lymph falls until it is the same as or even lower than the slowly rising concentration found in the plasma.[1,2] Similar results are obtained when the dye bromsulphthalein is used as the test substance. These observations suggest that jaundice produced by experimental extrahepatic biliary obstruction can be divided into an early and a late phase, and that the mechanisms involved in each phase may be fundamentally different.

EVIDENCE SUPPORTING THE REGURGITATION THEORY

As a matter of fact much of the experimental evidence put forward in support of the regurgitation theory is of an indirect or doubtful nature and therefore not entirely convincing. Thus the appearance in the plasma during biliary obstruction of substances which have apparently been modified by the hepatic cell (such as direct-reacting bilirubin, bromsulphthalein II and III,[3] etc.) or the occurrence in the hepatic and thoracic lymph of substances at concentrations which are higher than at any other site within the body except the biliary tree,[1] do not of themselves show that the test substances have ever entered the bile channels, although they may have been in the hepatic cells. Again, the histological demonstration in autopsy material of ruptured bile canaliculi in obstructive jaundice,[4] once presented as strong, if not conclusive, evidence of regurgitation, has not been borne out by biopsy specimens.[5] Hanzon[6] has reported observing direct leakages of bile from the canaliculi to the sinusoids, in the living animal, but his observations have been questioned by Grafflin and Chaney[7] using a similar technique, who were unable to find these leakages. The continuation of bile secretion during early biliary obstruction which has been both observed[6,7] and inferred,[8] demands the reabsorption of water and of biliary constituents (since their concentrations are in general not increased by obstruction) only if the volume secreted exceeds the extra capacity of the dilated biliary system.

THE USE OF DYES TO ESTIMATE VOLUMES WITHIN THE BILIARY SYSTEM

Recently some of the work carried out in this department has been directed towards a better understanding of the functioning of the biliary system in experimental animals during short periods of biliary obstruction, periods too brief to cause any detectable change in the function of the excretory mechanism. Suitable dye substances, known to be non-toxic and to be excreted predominantly in the bile, have been used in these studies mainly as exogenous markers. These dyes apparently belong to Brauer's class B substances,[9] since the concentration found in the bile may be many times that in the plasma. For this and other reasons an active secretory mechanism is thought to be involved and, since the excretion of these dyes shows many similarities to that of the bile pigments, it seems probable that they may share, at least in part, a common excretory pathway.

Dyes have been largely used in these studies to estimate the volume of the original contents which are still present in the rat biliary tree at any given time after injection of the dye.

The method of estimation may be explained briefly in the following manner: Let us for a moment consider the bile system to be a single tube with secretion occurring at one end, where the hepatic cells are situated, and bile leaving the tube at the other end to enter the duodenum (Fig. 1). Secretion of a marker dye into the tube theoretically permits the calculation of the internal volume of the tube, since this should equal the volume of clear (dye-free) bile collected between the time of injection and the appearance of the dye at the other end, i.e. the volume of clear bile pushed out of the tube in front of the advancing column of dye. When we do this in practice, collecting the excreted bile in successive small fractions, each of which is analysed separately, we find that the transition from clear bile to bile containing a high concentration of dye follows a sigmoid curve (Fig 2).

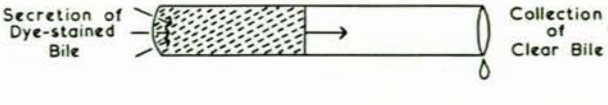

Fig. 1. Diagram showing the principle of the measurement of the volume of the biliary tree. Theoretically this is equal to the volume of clear bile pushed out of the tube by the advancing column of dye-stained bile.

There are a number of reasons why this should be so, the most important being the tree-like structure of the

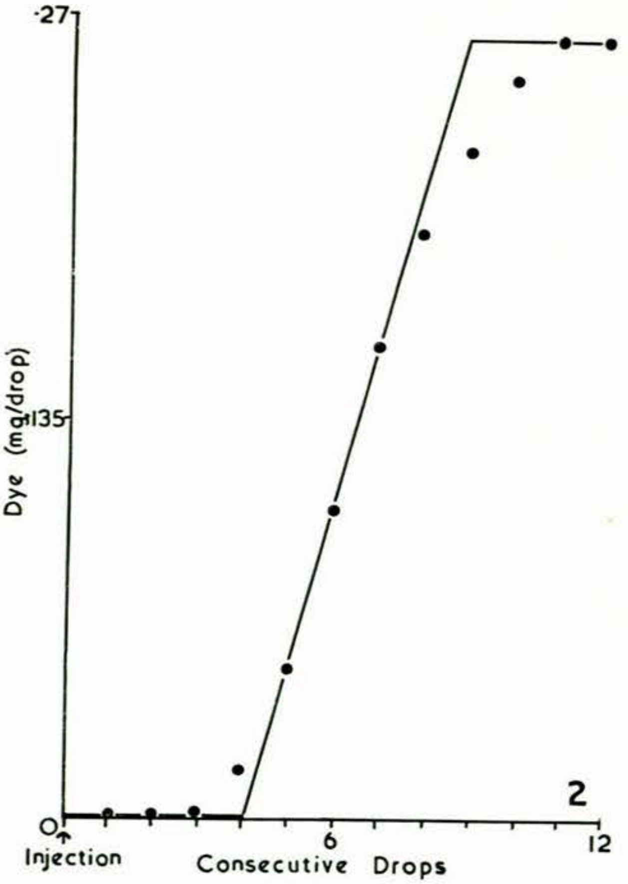

Fig. 2. Collection and analysis of consecutive drops of bile following an intravenous injection of dye shows that the rise in the dye concentration follows a sigmoid curve. The termination of the clear bile is not sharply defined as shown in Fig. 1, and the calculation of the volume of the biliary tree is accordingly complicated.

biliary system (as opposed to a single tube) and the probable occurrence of laminar flow within the narrow bile passages. From this it is apparent that the required volume is not given by the initial volume of clear bile collected. But as has been pointed out elsewhere,[10] the sigmoid curve is regular (inasmuch as probit transformation gives a reasonably straight line) and the other probable errors appear to be small, so there is reason to believe that the required volume may be obtained with some accuracy by measuring the volume of bile collected between the time of injection and the time when the dye concentration is half the maximum ultimately attained. This will be true only (*a*) if secretion of the dye occurs solely at the periphery of the biliary system, which in practice means that the hepatic cell rather than the ductular epithelium is the site of secretion;[6,7,11] and (*b*) if sufficient dye is injected to saturate the secretory mechanism.

Injections of very small quantities of dye have also been used in these studies because in this case the concentration of dye in the bile rapidly falls to a level which permits repeated experiments to be carried out on the same animal. But in this case the required volume is best given by the volume of bile collected between the time of injection and the time of appearance of the bile which contains the highest concentration of dye. The reasons for this procedure, and the evidence for the probable accuracy of these methods in general, will be given in detail elsewhere.

THE USE OF DYES TO DEMONSTRATE REGURGITATION

To return to the problem of biliary regurgitation. One way of demonstrating that regurgitation is taking place is by showing that the original contents of the biliary tree are removed and replaced by fresh bile during obstruction of the common bile duct; this may be shown in the following way: The common duct is obstructed for a period of time sufficient for full dilatation of the biliary tree to take place, after which no further secretion of bile can occur as long as the obstruction is maintained, unless either further dilatation takes place or some of the original contents are removed, i.e. when regurgitation occurs. At this stage a known quantity of dye is injected intravenously, and at the same time the obstruction is released. The bile is collected drop by drop, the dye content estimated, and the maximum volume of clear bile which the distended tree can hold is calculated in the manner already described. The experiment is then repeated in the same way, except that the obstruction is not released at the time of injecting the dye, but is continued for a further period of time until a steady state is reached with regard to secretion and reabsorption of dye, after which the obstruction is released and the volume of clear bile remaining in the system is calculated.

The results of experiments carried out in this way both with rats[10] and with cats (unpublished) showed that most of the clear bile originally present in the distended biliary tree was removed and replaced by dye-stained bile during the period of obstruction following the injection. The possibility of the undetected secretion during obstruction of a very small volume of bile containing a high dye concentration, and without an equivalent volume of bile being reabsorbed, may be ruled out since (*a*) the hepatic cell is limited in its ability to concentrate dyes and maximum concentration is attained in both experiments, and (*b*) it is found that the intrabiliary pressure is not significantly altered during the secretion of the dye. There can be little doubt therefore that in these experiments clear bile present in the biliary tree is reabsorbed during obstruction of the common bile duct and replaced by bile containing a high concentration of dye.

THE ROUTE OF REGURGITATION

When we turn to consider the route of regurgitation we find that there are many possibilities. In addition to a lymphatic return suggested by the appearance of biliary excretion products in the thoracic and liver lymph,[3] a direct return of bile to the blood from ruptured canaliculi has been described,[6] while at one time the ducts of Hering were singled out on morphological grounds for special consideration.[12] Furthermore the bile ducts and ductules of the portal canals with their associated vascular plexus of uncertain function must also be considered as potential sites of return flow.

With regard to the part played by the liver lymphatics, some experiments performed on dogs — and hitherto unpublished — will be briefly described here. They concern the concentrations of the dye bromsulphthalein found in the plasma after a single intravenous injection.

Fig. 3 shows the type of excretory curve usually found with normal dogs when the log. of the dye concentration in the plasma is plotted against the time following the injection.

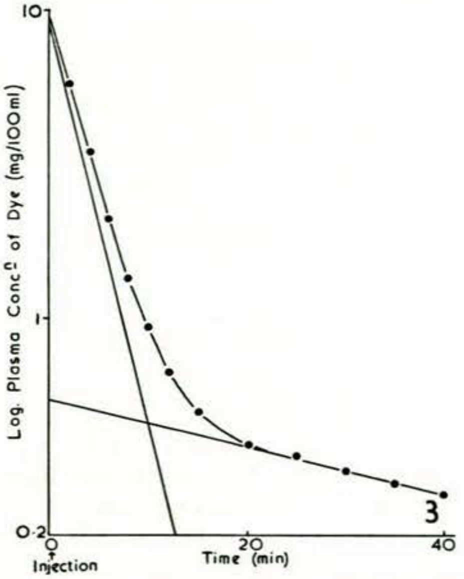

Fig. 3. The log. plasma concentration of dye plotted against time following a single intravenous injection in a dog. The straight lines represent an analysis of this curve into two exponential components.

This curve may be described with some accuracy as the sum of two exponentials, one being dominant in the early stages, but showing a rapid attenuation, and the other attenuating slowly and so becoming ultimately dominant.[13] Fig. 4 shows the graph obtained with the same animal after ligation of the common bile duct and removal of the gallbladder some 30 minutes before repeating the experiment.

It can be seen that the initial part of the curve is essentially similar to Fig. 3, but that after a few minutes the dye concentration in the plasma rises to a new level which is maintained for the rest of the experiment. The time of this increase coincides closely with the time of appearance of dye in the bile following a single injection in the normal (non-obstructed) animal. It would therefore appear that the rise in the plasma concentration of the dye seen in animals with experimental biliary obstruction is the result of an 'auto-injection' of dye from the biliary tree, i.e. of regurgitation of biliary contents to the vascular system.

This view is supported by a third experiment in which the gallbladder is again removed, but where the common bile duct is inserted into the inferior vena cava. The bile pressure is sufficient to prevent blood passing into the bile channels, while the blood pressure in the inferior vena cava is insufficient to prevent the normal flow of bile. When dye is injected intravenously into this preparation the resulting plasma concentrations are found to follow closely the curve obtained after obstruction of the common bile duct and shown in Fig. 4.

Finally, a preparation was used in which the gallbladder was removed, the common bile duct ligated and the thoracic lymph duct cannulated in the thorax. This procedure required the opening of the thoracic cage and hence the use of a respiratory pump which was carefully adjusted. Following the intravenous injection of the dye the plasma concentration again followed a course similar to that shown in Fig. 4 despite the drainage of the thoracic lymph. Analysis of this lymph showed that the dye concentration, as expected, soon became many times higher than that found in the plasma.

The high lymph-to-plasma ratio occurring in the first few hours following ligation of the cystic and common hepatic ducts is well known and has been demonstrated with regard to bile acids,[14] bilirubin[2,15] and bromsulphthalein.[2,15] But since raised plasma concentrations of these substances are still found when the thoracic or liver lymphatics are ligated, it follows that some other route of regurgitation is available, and reminds us that the significance of the part played by the liver lymph in regurgitation depends not only on the concentrations of substances contained therein, but also on the volume of lymph produced. This vital information is omitted from many accounts, as are also experimental controls with which to compare the plasma concentrations recorded.

Mayo and Greene[14] measured the plasma rise of bile acids after cholecystectomy and ligation of the common bile duct in dogs, and showed this to be very little altered by ligation or drainage of the thoracic duct. Their observations are in agreement with the experiments already mentioned where, following an intravenous injection, the concentrations of bromsulphthalein in the plasma of dogs with ligated cystic and common bile ducts were not found to be significantly altered by cannulation of the thoracic duct. The quantity (as opposed to concentration) of bromsulphthalein found in the lymph collected was small; thus in one experiment following a single injection of 50 mg. of bromsulphthalein, only 0·96 mg. was found in the 9·9 ml. of lymph collected during the next hour, a quantity sufficient to raise the plasma concentration by only about 0·2 mg. per 100 ml. even if injected all at once. It would appear from these experiments that the hepatic lymph plays an insignificant part in regurgitation during the first hour following complete biliary obstruction.

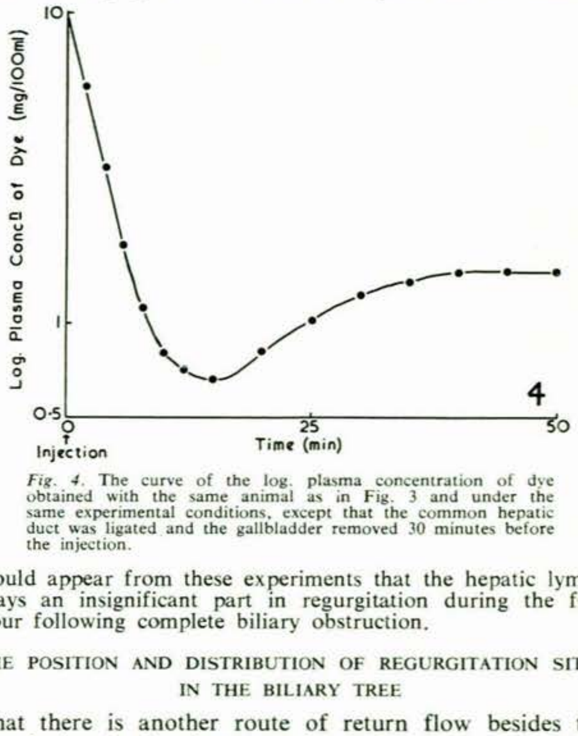

Fig. 4. The curve of the log. plasma concentration of dye obtained with the same animal as in Fig. 3 and under the same experimental conditions, except that the common hepatic duct was ligated and the gallbladder removed 30 minutes before the injection.

THE POSITION AND DISTRIBUTION OF REGURGITATION SITES IN THE BILIARY TREE

That there is another route of return flow besides the lymphatics is therefore not in doubt, although as has already been mentioned neither the pathway nor the mechanism of this 'direct' route is known with certainty. An attempt has been made, however, to define the position and distribution of the sites at which the bile leaves the biliary tree during early regurgitation, using the dye method of volume measurement previously described. In these experiments the volume of residual clear bile present in the system is measured at different time intervals following an intravenous injection of dye.

Fig. 5 shows the curve obtained when these values are plotted against the time interval on ordinary graph paper.

The final volume of residual clear bile in these experiments was, after subtraction of the cannula dead space, something less than 10% of the total volume of the distended bile tree. In other words, about 90% of the bile present in the distended system is concerned in the process of regurgitation, and it has therefore been suggested that the bile channels of the portal system must play a part in this process, on the grounds that it is unlikely that the bile canaliculi contain 90% of the bile present in the distended tree.[10]

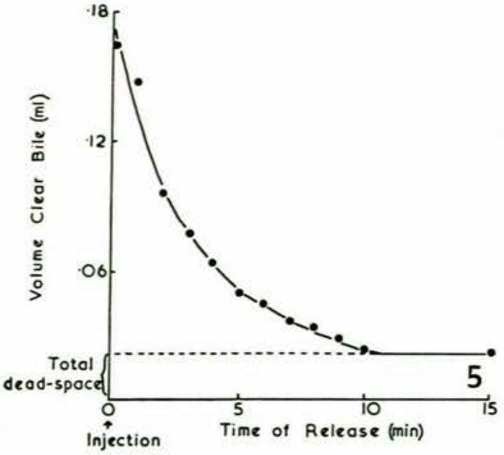

Fig. 5. The decline in the volume of the residual (clear) bile present in the rat biliary tree at different time-intervals following a single intravenous injection of dye.

Since the secretion of dye occurs at the periphery of the biliary tree, it follows that the relative distribution of the reabsorption sites (as well as their position) may be deduced from the rate of decline of the volume of residual clear bile. Thus, if these sites had been situated at the lower end of the biliary tree (i.e. at the opposite end to the site of secretion) then the rate of decline would have been arithmetical and the graph shown in Fig. 5 would have been a straight line. In fact it is found that when log. (residual volume minus total dead space) – where total dead space = instrument dead space plus residual dead space – is plotted against the time interval between the injection and the release of the obstruction, a straight-line relationship is found (Fig. 6). This indicates that the reabsorption sites are probably uniformly distributed throughout all the biliary tree concerned in reabsorption, i.e. throughout all the biliary tree except a small zone immediately adjacent to the cannula which is the residual dead-space volume and which probably includes little more than the upper parts of the extrahepatic ducts.

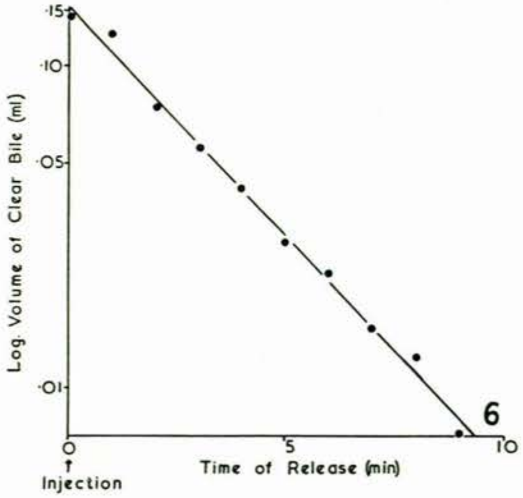

Fig. 6. The values shown in Fig. 5 corrected for total dead-space volume and plotted on a log. scale. The decline in this volume of residual bile is exponential with time.

Since the secretion of dye produces no significant change in the volume contained in the distended bile tree, it follows that the initial slope of the curve in Fig. 5 represents the rate of total reabsorption, and if obstruction of the common bile duct for a short period of time has no effect on biliary secretion, as has been suggested,[8] then this total reabsorption rate should be equal to the secretion rate. In fact, when the reabsorption rate is measured in this way, it is always found to be slightly faster than the control secretion rate. Thus, in the example shown, the reabsorption rate is about 3 drops of bile per minute, while the secretion rates, measured between each experimental procedure, vary between 2 and 2·5 drops per minute. Since from first principles there is no reason to suppose that secretion is increased by obstruction, this apparent increase in the rate of flow is thought to be the result of imperfections in the method of measuring, pos

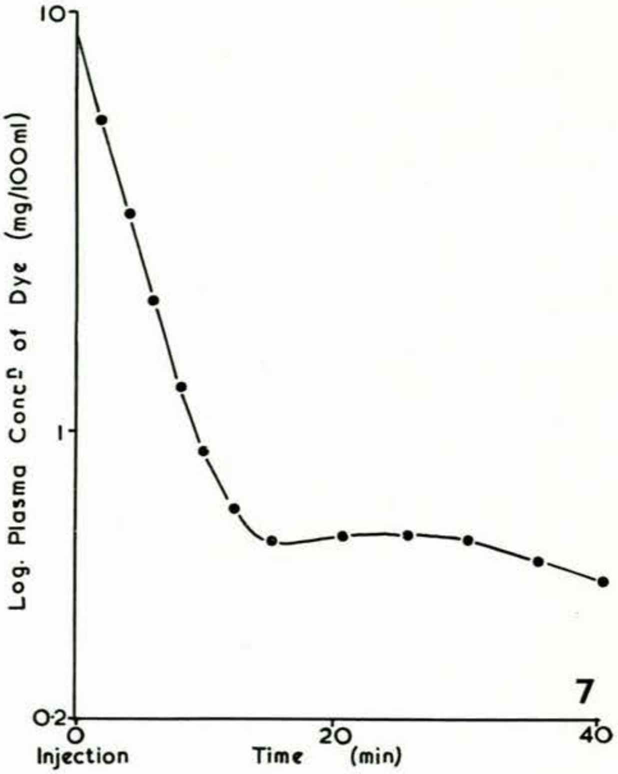

Fig. 7. Curve commonly found with dogs when the log. plasma concentration of dye is plotted against time following a single intravenous injection (*cf* Fig. 3).

sibly owing to the forward diffusion of dye through the bile.

But let us suppose for a moment that these measurements are substantially correct. After all, the 'secretion rates' measured are really the rates of bile flow through the cannula. and it is possible that some reabsorption of the contents occurs during the passage of the bile down the bile channels. This suggestion receives some support from another 'imperfection', this time in the plasma dye curves found following a single intravenous injection made into the normal (non-obstructed) animal. Fig. 3 shows that these curves may be described as the sum of two exponentials, but careful examination of many curves of this type after doses of 3-5 mg./kg. of bromsulphthalein shows that the falling plasma concentration is arrested, and often temporarily slightly reversed, about 20-30 minutes after the time of injection of the dye (Fig. 7). The effect seen is small but is a common finding. It is certainly due to re-entry of dye into the plasma, but is not due to enterohepatic circulation, since the effect is observed during cannulation of the bile. The most likely source of this returned dye would appear to be bile. It therefore seems possible that while the mechanisms of bile production are mainly those of secretion and diffusion, reabsorption may modify this initial fluid during its passage through the bile channels.

SUMMARY

A method of measuring the volume of the original contents of the biliary tree remaining at any time following an injection of dye is briefly discussed. A review is given of the application of this method to different aspects of early biliary obstruction, together with certain other experimental observations.

The results confirm that secretion of bile continues during short periods of biliary obstruction and is accompanied by a regurgitation of biliary contents to the vascular system. This return flow apparently involves most, if not all, of the intrahepatic biliary system, and the reabsorption sites are found, within the limits of accuracy of the method, to be distributed in a uniform manner throughout the biliary tree. While the route of return of biliary constituents from the bile channels to the blood plasma is not known, reasons are given for believing that the hepatic lymph, which is known to carry some of the regurgitated bile constituents under certain conditions, is neither the only nor the main route of return.

This experimental work has so far been restricted to the early phase of biliary obstruction. It is possible, and even probable, that the mechanisms investigated are fully functional only in the first few hours of complete biliary obstruction.

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