Sources of proteins in human bile

BARBARA M MULLOCK, LYNNE J SHAW, B FITZHARRIS, JANE PEPPARD,
M J R HAMILTON, M T SIMPSON, T M HUNT, AND R H HINTON

From Robens Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, Surrey, Section of Tumour Immunology, Institute of Cancer Research, Belmont, Sutton, Surrey, Department of Microbiology, Bristol Royal Infirmary, Bristol, Westminster Hospital, Dean Ryle Street, London, and St Luke's Hospital, Guildford, Surrey

SUMMARY The proteins of 46 human bile specimens, collected by several different routes have been studied by crossed immunoelectrophoresis, by rocket immunoelectrophoresis and by radioimmunoassay. The results were analysed by plotting the variation in the bile:plasma ratio of particular proteins against molecular weight and by examination of the correlation between the concentrations of different proteins in the biles of different patients. Our results show that the majority of human bile proteins derive from plasma although bile specific proteins are always present. The majority of plasma proteins appear to enter bile by a 'sieving' mechanism which results in an inverse relationship between the bile:plasma ratio and the molecular weight. In addition there was a very high degree of correlation between the biliary concentrations of α_2 -macroglobulin, IgG, haptoglobin, haemopexin, albumin, prealbumin, and orosomucoid. A number of other proteins namely thyroxine binding globulin, GC globulin and α_2 HS-glycoprotein appeared in bile at concentrations greater than those expected if entry is by the sieving mechanism. These three proteins, however, are of rather low molecular weight and the reason for the lack of correlation appears to be individual variation in the 'pore size', presumably reflecting variation in the porosity of tight junction between hepatocytes. Although the majority of human bile proteins would appear to enter bile by a molecular weight-dependent pathway, four proteins, namely secretory IgA, IgM, haemoglobin and caeruloplasmin, showed significant deviation from the predicted relationship and probably enter bile at least partly by transport across cells. The concentration of β_2 -glycoprotein I was also much greater than expected from its molecular weight. The reason for this is not yet clear but may well reflect a very efficient and specific transport mechanism.

The principal proteins of rat bile,¹ are, with the exception of secretory component, derived from plasma.²⁻⁴ Transfer from plasma to bile occurs both by transport across hepatocytes and by sieving across tight junctions (paracellular transport⁵), the former being quantitatively more important. In addition minor amounts of biliary protein are dissolved from the bile-facing cell surfaces or are derived from lysosomal discharge. These results are consistent with previous data published by Dive and colleagues⁶ on the composition of dog bile. Examination of other species such as chickens,⁴ pigs,⁴ sheep^{4,7} and guinea pigs^{4,8} does, however, show

that there can be marked species variation in the importance of the transcellular pathway. In the species mentioned, the bulk of biliary protein probably derives from the paracellular pathway, whereas in dogs, cats and rabbits,⁴ as in rats, the bulk of the protein derives from transport across cells. The data of Dive and Heremans⁹ suggest that in human bile the bulk of protein probably reaches bile by the paracellular pathway. Since that time, however, many workers have found that human bile is rich in secretory IgA¹⁰⁻¹⁴ and that secretory IgA¹⁴⁻¹⁸ and free secretory component^{18,19} accumulate in serum in liver disease.

In rats, and other species, polymeric IgA is quantitatively the most important protein transferred from plasma to bile by passage across hepa-

Address for correspondence: Dr B Mullock, Toxicology Unit, The Robens Institute, University of Surrey, Guildford, Surrey GU2 5XH.

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toocytes.⁴ It would appear that similar transport must occur in man although the precise site and quantity of transfer has been the subject of considerable debate.^{4 9 20-23} In rats, moreover, it seems clear that IgA is not the only protein which can be transferred from plasma to bile by passage across hepatocytes; this would also appear to be the route of transfer for haemoglobin, which appears in bile as a haptoglobin-haemoglobin complex^{24 25} and possibly for other proteins.²⁵ The main systematic studies on human bile proteins are those carried out by Dive and Heremans in 1974⁹ in which only five proteins were investigated and the study of Delacroix *et al*²⁶ which concentrated on biliary immunoglobulins. We have, accordingly, re-investigated the protein composition of human bile using specimens drawn by a variety of techniques and using techniques which enable us to study a wide variety of different proteins.

Methods

MATERIALS

Monospecific antisera against human serum proteins were, when available, those prepared by Dako Immunoglobulins, (Copenhagen, Denmark) and supplied through Mercia Brocades (Byfleet, Surrey). Other antisera were purchased from Nordic Immunological Laboratories (Maidenhead, Berks), Seward Laboratories (London SE1) or Behringwerke AG (Marburg, West Germany). Anti-human bile antiserum was prepared in rabbits maintained in the University of Surrey animal house using the injection schedule described in earlier studies.²⁷ Only 'normal' biles, as classified in paragraph 2 of the results section, were used in the antigen-mixtures injected.

Twelve bile samples from six patients, five of whom had undergone cholecystectomy were obtained by endoscopic retrograde choledochopancreatography (ERCP) and given to us by Dr Mary Chapple, Middlesex Hospital.

Six bile samples were obtained by T-tube drainage after cholecystectomy and provided by Dr Parveen Kumar, St Bartholomews Hospital and one sample from St Georges Hospital.

Thirteen samples were obtained by needle aspiration from the biliary tract at laparotomy from patients not known to have hepatobiliary disease, and were given to us by Mr Tim Cooke, Southampton General Hospital.

Bile from subjects without hepatobiliary disease was obtained by aspiration from the duodenum. One sample was a pool of duodenal juice from 12 normal volunteers and the gift of Dr John Thornton, Bristol Royal Infirmary, and two were

from patients being investigated at Addenbrookes Hospital, Cambridge and were provided by Dr Mary Calladine.

Fourteen matched serum and bile samples from 13 patients were obtained from patients undergoing T-tube drainage at St Lukes Hospital, Guildford after either cholecystectomy or after biliary obstruction resulting from carcinoma of the head of the pancreas. Five matched bile and serum samples from four patients were also obtained at Westminster Hospital by aspiration of gall bladder contents within five minutes of surgical removal.

When more than one sample was obtained from a single patient, only one specimen was used in the main analysis. A selected series of test were carried out on the remaining specimen to determine whether there were significant alterations with time of collection.

Bile proteins were analysed by crossed immunoelectrophoresis according to Axelsen *et al*.²⁸ Quantitation of proteins in serum and bile was carried out by rocket immunoelectrophoresis.²⁸ In all cases a range of dilutions of, as appropriate, normal human serum or the matched patient serum was run on the same plate. Results from bile samples were only accepted if they lay within the range of serum standards. Haptoglobin:haemoglobin complexes were estimated by rocket immunoelectrophoresis against anti-human haemoglobin. This procedure is valid, because results presented below show no free haemoglobin in any serum or bile specimen.

Secretory IgA was assayed by an adaptation¹⁴ of the radioimmunoassay procedure of Peppard.²⁹ The antiserum was an anti-(human secretory component) made by methods similar to those of Socken and Underdown.³⁰ Briefly, human secretory component was prepared by passing spun human milk through a column containing human polymeric IgA immobilised by binding to cyanogen bromide activated Sepharose. Bound secretory component was eluted with 1.5 M potassium thiocyanate at 4°C. Antiserum to this preparation, raised in rabbits, was purified by absorption with monomeric IgA, lactoferrin and normal human serum. The resulting antiserum was specific for secretory IgA and free secretory component. Free secretory component, measured by crossed immunoelectrophoresis with a split antibody containing gel,²⁸ however, was detected in only a minority of bile samples and never amounted to more than 15% of the material reacting with the antiserum.⁴ The secretory IgA used as a standard was a mixture of dimeric and trimeric secretory IgA prepared from human milk by a combination of ion exchange chromatography and gel filtration on

Ultrogel AcA22 and AcA34 (LKB Instruments, Croyden, Surrey).

Statistical analysis of the results was carried out using the 'correlation' and 'regression' procedures of the MINITAB statistical package Release 82.1 (University of Pennsylvania Statistical Laboratory) implemented on the University of Surrey's Prime Computer.

Results

The means of collection of bile specimens and the provisional diagnosis of the patients are indicated in Table 1. Two dimensional agarose gradient polyacrylamide gel electrophoresis of human biles showed a protein pattern similar to that of human serum although with a notable depletion of high molecular weight proteins (not illustrated). In particular bile specimens from persons bearing the Hp_2^a haptoglobin gene showed a similar 'ladder' of bands caused by haptoglobin monomers as did serum specimens from such individuals. Crossed immunoelectrophoresis against antihuman serum and an antiserum raised against human bile showed that the majority of human bile proteins reacted with anti-human serum (Fig. 1) although some bile-specific proteins were consistently present.

Bile samples were collected from 46 different patients. Study of the concentrations of particular proteins in the initial batch of 29 samples showed that the bile specimens could be divided into three groups. In the majority of specimens, there was, for major plasma proteins such as IgG, transferrin and albumin, a linear relationship between the ratio of the biliary and normal plasma concentration and the reciprocal of the molecular weight

as has already been described by Dive and Heremans (Fig. 2a). Significant numbers of patients, however, had very large amounts of high molecular weight proteins in their bile (Fig. 2b). In these patients there was little change in the bile-plasma ratio with molecular weight, which is consistent with massive leakage of plasma into bile. These were classified as plasma-rich biles. There appeared to be a clear cut distinction between the two groups – whereas the range of α_2 -macroglobulin in the 23 normal biles was 0–1.9% of the plasma concentration, that in the five plasma-rich biles was 5.0–6.8% of the plasma concentration. Other patients had practically no proteins present in their biles (classified in Table 1 as protein-poor biles). There was no correlation between the amount of protein in the bile and either the means of collection or the provisional diagnosis (Table 1). No systematic differences could be observed between biles obtained by T-tube drainage and those obtained by what seemed to be the least invasive procedure, withdrawal during laparotomy. Very similar patterns were obtained when biles from the same patient were examined at different times after cholecystectomy with T-tube drainage or after endoscopic exploration of the bile duct.

Although the biliary concentrations of the majority of proteins which we examined obeyed the reciprocal relationships with molecular weight, as is clear from Fig. 2, six proteins, namely IgA, caeruloplasmin, haptoglobin, haemoglobin, orosomucoid and β_2 -glycoprotein I, were present in bile at concentrations significantly greater than would be predicted from the concentrations of proteins such as albumin. Of these proteins a considerable proportion of the IgA was combined with

Table 1 Source and nature of human bile samples examined in the current study. Normal, 'plasma rich' and 'protein poor' biles are defined in paragraph 2 of the results section. In summary plasma rich biles are those in which the concentration of α_2 -macroglobulin was greater than 1% of its serum value while 'protein poor' biles are those in which the concentration of albumin was less than 0.5% of its plasma value. 'Normal' biles showed a linear relationship between the concentration of major serum proteins and the reciprocal of the molecular weight. 'Other' biles showed anomalous features some of which could have been caused by proteolysis

	Carcinoma of pancreas	T tube drainage after*					Duodenal aspiration [†]	Gall bladder content
		Cholangioma	Cholecystectomy	Endoscopic drainage	Aspiration from bile duct			
Normal	1	2	5	5	9	0	2	
Plasma-rich	2	0	1	1	4	0	1	
Protein poor	1	0	3	0	0	0	0	
Other	2	0	0	0	0	3	0	

* Four samples were supplied without summaries of clinical notes.

[†] These biles contained little protein other than secretory IgA. This could be explained by partial aspiration of gastric contents or, more likely, by partial proteolysis.

Fig. 1 Pattern obtained after crossed immuno-electrophoresis of 5 μ l of a human bile specimen. Electrophoresis in the second dimension was against a split antibody-containing gel containing in the lower part 5% anti-human serum (Dako, Copenhagen) and in the upper part 5% anti-human bile. It should be noted that, because of cathodic drift of IgG, the precipitation lines indicated by arrows which are due to proteins reacting with anti-(human bile) but not with anti-(human serum) appear somewhat below the junction of the two gels.

secretory component while haemoglobin was present in the form of haptoglobin:haemoglobin complexes (Fig. 3).

For the purposes of mathematical analysis, the biliary concentration relative to normal plasma of the high molecular weight protein α_2 -macroglobulin may be taken as an indication of the extent of direct leakage. The differences between the relative concentrations of albumin and α_2 -macroglobulin may be taken as an indicator of the amount of protein transported by sieving. We accordingly carried out a statistical analysis of the variations of concentrations of proteins in different biles in relation to these parameters and to the concentration of secretory IgA. The results (Table 2) show that most of the variation in the concentrations of IgG, transferrin, haemopexin, pre-albumin and orosomucoid correlate with variations in albumin and α_2 -macroglobulin concentrations whereas the variations in IgA, haptoglobin, haemoglobin, caeruloplasmin, thyroxine-binding globulin and β_2 -glycoprotein I show no such correlation, although neither do they correlate well with

concentrations of secretory IgA in the bile. The plasma concentration of several of these proteins is known to change in disease, however, and we therefore felt that some of the variation in biliary concentrations could be because of variations in the concentrations in plasma. We accordingly carried out a second series of studies on patients where we were able to obtain plasma samples drawn at approximately the same time as the sample of bile.

When matched specimens were received, we initially measured the concentrations of α_2 -macroglobulin and albumin in plasma and bile. The two specimens in which α_2 -macroglobulin in bile was greater than 1% of the plasma concentration or those in which the albumin was either less than 0.5% of plasma concentration or similar to the α_2 -macroglobulin were rejected as 'abnormal'. Analysis of the relative concentration of proteins in bile and plasma (Fig. 4a) then showed a linear relationship between the relative biliary concentration of proteins and the reciprocal of the molecular weight for the higher molecular weight prot-

eins α_2 -macroglobulin, IgG, haptoglobin and transferrin. The lower molecular weight proteins albumin, prealbumin and orosomucoid deviated markedly from the line. Examination of individual

Fig. 2 Relationship between the concentration of proteins in bile, expressed as a ratio of the concentration of the same protein in normal human serum, to the inverse of the molecular weight of the protein. (a) Results from 23 'normal' bile specimens. (b) Results from 5 bile specimens containing large amounts of plasma proteins (plasma rich biles). The bars extend one standard error on each side of the mean. The number in brackets after the name of each protein shows the number of patients in which that protein was actually measured. Thyroxine-binding globulin could only be measured in biles relatively rich in plasma proteins. The results have accordingly been scaled down by the ratio of the relative albumin concentration in these biles to the relative albumin concentration in all bile specimens.

results (Table 3) showed that this deviation was because of the influence of two bile specimens (numbers 6 and 8). Omission of these results resulted in a smooth curve (Fig. 4b) with five proteins, namely secretory IgA, IgM, haemoglobin, caeruloplasmin and β_2 -glycoprotein I deviating from the line.

Statistical analysis of the data (Table 4) showed that the concentrations of IgG, IgM, orosomucoid and prealbumin correlated well with the degree of 'sieving' assessed as the difference between the relative concentration of albumin and α_2 -macroglobulin. There was also a significant correlation between the concentrations of haemoglobin (present as haptoglobin:haemoglobin complexes) and the concentration of secretory IgA and a weaker correlation between the relative concentrations of secretory IgA and caeruloplasmin.

Discussion

Our results indicate that the majority of the proteins of human bile derive from plasma although significant amounts of bile-specific proteins are present. The routes by which individual proteins reach bile may, in some cases, be deduced by further con-

Fig. 3 Rocket immunoelectrophoresis of human bile specimens and dilutions of a 'normal' human serum against: (a) A split antibody-containing gel with, in the lower part, 2% anti-human haptoglobin and, in the upper part 1.5% anti-human haemoglobin. (b) A split antibody-containing gel with, in the lower part, 1.5% anti-human haemoglobin and, in the upper part, 2% anti-human haptoglobin.

Table 2 Correlation between amounts of proteins in individual biles relative to the concentrations in normal human plasma and the relative concentration of (1) α_2 -macroglobulin [α_2M]', taken as a measure of the leakage of whole plasma into bile, (2) the difference between the relative concentrations of albumin [Alb]' and α_2 -macroglobulin, taken as a measure of sieving of proteins across tight junctions and (3) the relative concentration of secretory IgA [sIgA]'. The regression equation fitted was:

$$[X]' = a + b[\alpha_2M]' + c([Alb]' - [\alpha_2M]') + d[sIgA]'$$

where [X]' is the concentration of the protein under study. The results presented below show the calculation regression coefficients a, b, c and d and the % of the variation of [X]' explained by the three regressors, entered in the order given and the unexplained variation.

Protein	No	a	b	c	d	% of sum of squares explained by			Unexplained variation %
						[α_2M]'	[Alb]' - [α_2M]'	[sIgA]'	
IgG	26	0.057	0.916*	1.09*	-0.0003	58.6	35	0.5	5.9
Orosomucoid	27	4.33*	2.38*	1.75*	0.0005	59.2	14.3	0.2	26.3
Prealbumin	27	0.75*	0.81*	0.23	0.0004	77.8	5.7	1.5	15.0
Transferrin	25	0.337	0.49*	0.126	0.0008*	72.0	5.6	2.3	16.5
Haemopexin	26	1.27	0.99*	0.87	-0.0001	44.4	12.3	0	43.1
IgA (total)	25	4.49*	-0.007	0.593	0.0018	8.7	6.2	5.8	85.1
Haptoglobin	24	8.83	-1.10	1.54	0.003	0	12.1	2.8	85
Haemoglobin	26	2.4	1.19	1.56	0.0027	36.8	11.2	2.7	55
Caeruloplasmin	25	2.94*	0.71	-0.26	0.0003	32.6	0.4	0.4	66.5
Thyroxine-binding globulin	16	5.28	0.39	0.85	0.0009	0.15	2.4	15	95.9
β_2 -Glycoprotein I	17	7.56	0.46	0.58	0.0009	0	3.3	4.1	96.3

An asterisk against any of the regression coefficients indicates it to be significantly different from zero, ($p < 0.05$). No = number of biles studied.

sideration of the data. In humans, as in rats, plasma proteins may be transferred to bile either by transport across hepatocytes or by the trans-cellular pathway. In addition a minority of the present bile specimens, although not visibly tainted with blood, appeared to show evidence of direct leakage of total plasma into bile for they contained a high concentration of plasma proteins and the

concentration of particular proteins, relative to plasma, showed little dependence on molecular weight. It seems likely that such direct leakage occurs between damaged hepatocytes or bile duct lining cells for although the sinusoidal endothelium will prevent passage of red blood cells it provides no significant barrier to the passage of plasma proteins. Biles which appear to show such direct

Table 3 Concentration of proteins in 10 human specimens given as a percentage of the concentration of the same protein in plasma

	Patient									
	1	2	3	4	5	6	7	8	9	10
Albumin	0.8	1.5	1.3	1.2	2	19.8	1.5	19.4	1.9	1.5
α_2 -Macroglobulin	0.6	0.4	0.5	0.6	0.8	0	1.3	0.8	0.2	0.5
IgG	0.7	0.6	0.6	0.6	1.7	1.7	2.1	3.4	1.0	2.3
IgM	7.5	2.3	1.9	8.9	9.4	13.6	2.7	58	0	20
Secretory IgA	1176	110	170	799	167	371	1673	439	211	823
Orosomucoid	1.6	2.3	1.8	4.4	4.3	23.4	9.2	14.5	3.6	2.9
Prealbumin	7.5	1.9	2.2	1.2	4.5	31.1	9.4	31.3	4.3	5.5
Transferrin	1.0	1.0	1.1	0.3	1.6	1.0	2.1	1.5	1.8	2.3
Haemopexin	1.8	7.4	0.7	2.1	3.8	0	1.9	0	3.6	3.3
Haptoglobin	0.4	0.6	0.5	0.8	1.4	0.5	7.0	0.8	0.6	1.3
Haemoglobin	3.1	0.8	0.3	5.7	0	0	25.2	0	0	15.8
Caeruloplasmin	1.7	1.8	1.7	0	1.8	2.3	6.3	0	1.2	4.7
α_2 HS-Glycoprotein	4.2	1.2	0.7	2.2	2.8	0	4.5	0	2.9	2.5
β_2 -glycoprotein	46.4	17	21.4	ND	ND	0	14.7	ND	ND	ND
GC-globulin	8.9	3.75	2.2	ND	ND	0	14.7	ND	ND	ND

Fig. 4 Relationship between the concentration of proteins in bile, expressed as a ratio of the concentrations of the same protein in plasma of the same patient, to the inverse of the molecular weight of the protein: (a) Results from all 10 specimens examined. (b) Results omitting the two biles (Table 3, Nos 6 and 8) with anomalously high albumin concentrations.

Table 4 Correlation between amounts of proteins in 10 individual biles relative to the concentrations in plasma from the same patient and the relative concentration of (1) α_2 -macroglobulin [α_2 -M]', taken as a measure of the leakage of whole plasma into bile, (2) the difference between the relative concentrations of albumin [Alb]' and α_2 -macroglobulin, taken as a measure of sieving of proteins across tight junctions and (3) the relative concentration of secretory IgA [sIgA]'. The regression equation fitted was:

$$[X]' = a + b[\alpha_2M]' + c([Alb]' - [\alpha_2M]') + d[sIgA]'$$

where [X]' is the concentration of the protein under study. The results presented below show the calculation regression coefficients a, b, c and d and the % of the variation of [X]' explained by the three regressors, entered in the order given and the unexplained variation.

Protein	a	b	c	d	% of sum of squares explained by			Unexplained variation %
					$[\alpha_2M]'$	$[Alb]' - [\alpha_2M]'$	$[sIgA]'$	
IgG	0.16	1.62	0.09*	0	13.3	53.3	0	33.4
IgM	-5.94	24.3	1.77*	0.0059	2.3	60.0	1.9	35.8
Orosomuroid	1.43	-1.19	0.87*	0.0036	3.4	80.1	4.1	12.4
Prealbumin	-0.66	2.12	1.53	0.0041*	1.7	94.7	2.0	1.53
Transferrin	1.04	0.52	0	0	10.9	0	0.2	88.9
Haemopexin	4.39	-0.12	-0.19	-0.0017	0.5	37.0	9.5	53.1
Haptoglobin	3.32-1.25	3.00	0.01	0.0015	58.9	0	9.1	32.0
Haemoglobin	-3.32	3.69	-0.14	0.0046*	39.7	2.4	29.7	28.2
Ceruloplasmin	1.0	0.06	0.03	0.0021	16.6	2.6	19.2	61.6
α_2 HS-glycoprotein	1.65*	-0.07	-0.12	0.0017	26.1	33.8	19.0	21.0

An asterisk against any of the regression coefficients indicates it to be significantly different from zero, ($p < 0.05$).

leakage have been excluded from the further analysis of results as have the minority which contained very little protein of any kind as in both cases it seemed likely that surgical procedures and/or the underlying clinical condition which necessitated them had seriously affected the composition of the bile. The remaining samples, while clearly not a random sample of a normal healthy population show a consistent pattern which allows analysis of the mechanisms which govern the protein composition of human bile. The pattern obtained is not the result of the influence of the underlying liver disease for samples obtained by aspiration from the bile duct after laparotomy for conditions not connected with liver disease showed a similar pattern to 'normal' bile obtained, for example, from drainage after cholecystectomy.

In the majority of bile specimens, however, the concentrations of major plasma proteins were markedly dependent on molecular weight. The pioneering studies of Heremans and his colleagues^{6,9} showed that both in man and in dogs the variation in concentration in bile of the major plasma proteins albumin, orosomucoid, α_2 -macroglobulin and IgG could be explained by sieving through 'pores' with a maximum effective radius of 12.7 nm in man and 11.3 nm in dogs. Our studies show that, in addition to the four proteins mentioned, the bulk of bile haptoglobin, transferrin and prealbumin reach bile by sieving. For these proteins, the ratio of biliary to plasma concentrations plotted against the reciprocal of the molecular weight for each protein gives a straight line. Moreover, their biliary concentrations correlate well with the concentrations of albumin and α_2 -macroglobulin in the same bile. The situation is less clear cut for a few other proteins, namely haemopexin, thyroxine-binding globulin, GC globulin and α_2 HS-glycoprotein. The mean concentration of these proteins in bile, relative to their concentrations in serum are approximately those expected from their molecular weights (shown in Figs. 2 and 4) but the bile/serum ratios in individual patients (presented in Tables 2 and 4) correlate badly with the bile/serum ratios of albumin and α_2 -macroglobulin. All these proteins are, however, lower in molecular weight than albumin and the variation could be explained if there is individual variation in the degree of sieving of low molecular weight protein. Plotting the serum/bile ratios presented in Table 3 in individual patients against the inverse of molecular weight suggested that this is indeed the case. These results suggest that in some individuals the permeability of the barrier between blood and bile increases sharply at molecular weights of less than 60 000. In the majority of patients upturn in the

curve is found at lower molecular weights, indicating that the permeability change normally occurs at a molecular weight of around or below 40 000. This suggests that the permeability of the blood-bile barrier is similar to that of the blood-urine barrier,³¹ which is likely because the permeability of tight junctions between hepatocytes probably governs the entry of proteins into bile in the same way as the permeability of tight junctions between podocytes governs entry of proteins into urine.³²

Thus the majority of bile proteins seem to derive from ultrafiltration of blood through the tight junctions between hepatocytes, the so called paracellular pathway.⁵ There are, however, several notable exceptions namely, haptoglobin:haemoglobin complexes, β_2 glycoprotein 1 and possibly caeruloplasmin. There is considerable evidence (see introduction) that secretory IgA is transferred into human bile by transport across cells although there is dispute as to whether transfer occurs across bile duct lining cells or hepatocytes. The low correlation between the bile:plasma ratios of total IgA and the bile:plasma or total (results not presented) levels of sIgA found in this study does, however, indicate that appreciable amounts of IgA enter bile independently of secretory component. This is consistent with the report²⁶ of significant amounts of monomeric IgA in human bile. In the present study we found that the concentration of haptoglobin:haemoglobin complexes was much greater in bile than would be expected from the molecular weight of these complexes. This is unlikely to be caused by bleeding into the biliary tree as the effect is most marked in bile in which there are minimal amounts of high molecular weight plasma proteins. Hence it seems that haptoglobin:haemoglobin complexes can be transferred across cells into bile as is the case in rats.²⁴ The significant correlation between the concentration of sIgA and haptoglobin:haemoglobin complexes in matched serum and bile specimens suggests that in humans, as in rats,²⁵ the two proteins are transferred in the same system. The concentration of β_2 -glycoprotein I in bile relative to its concentration in plasma was much greater than would be expected from its molecular weight. This protein is known to have marked membrane binding properties³³ and may be involved in lipoprotein metabolism³⁴ it is, therefore likely that the relatively high concentration of β_2 -glycoprotein I in bile reflects some form of active transfer. Caeruloplasmin may behave similarly, although the concentration in bile was only twice that expected from the molecular weight and the correlation with IgA was not statistically significant. Clearly it is theoretically possible that, as the

majority of plasma proteins are made in the liver³⁵ there is direct release of proteins into bile. Studies on the labelling kinetics of proteins of rat bile show uniform labelling of serum related proteins,³⁶ however, and it would appear that even secretory component, which is released only into bile, is directed first to the sinusoidal face of the cell and then returns across the cell in endocytic vesicles.³⁷ Accordingly it is unlikely that there is any direct secretion of proteins into bile.

The amount of IgM in bile is much greater than would be expected from its molecular weight, but in this case there was considerable correlation between the concentration of IgM and the degree of 'sieving' assessed as the difference between the relative concentrations of albumin and α_2 -macroglobulin. The high concentration of IgM in human bile is most probably because of its transfer across cells with secretory component as receptor. This theory is supported by the close correlation between the absolute levels of secretory IgA and IgM in the bile, is consistent with the small amounts of secretory IgM reported in some human biles³⁸ and with earlier work which has shown that rats may transport IgM from plasma to bile using secretory component acting as receptor.³⁹ Accordingly it would seem likely that a considerable part of the biliary IgM may have been carried along the same pathways as polymeric IgA.

Thus ultrafiltration across the junctional complexes supplies by far the largest proportion of human bile proteins while specific transport of particular proteins across cells, the major route of entry of proteins into bile in rats,²⁻⁴ is a less important contributor in the entry of proteins. Furthermore, it would appear that, at least in liver disease, there can be leakage of plasma into bile, presumably, as large amounts of haemoglobin are not present, this is either because of leakage from lymph into bile ductules or of damage to hepatocytes resulting in leakage into bile canaliculi from the space of Disse. Finally it must not be forgotten that quantitatively minor, although possibly functionally important proteins, such as the bile specific glycoproteins described by Svenberg,⁴⁰ appear in bile, although not in serum, and may well, like many biliary enzymes² derive from solubilisation of the proteins from the bile canalicular surface of hepatocytes.

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