THE DOMINATING MACROMOLECULAR COMPLEX OF HUMAN GALLBLADDER BILE

by

J. C. M. VERSCHURE AND P. F. MIJNLIEFF

with technical assistance from

MISS F. M. C. HOEFSMITT AND MRS A. E. NOOTER VAN DER HOEVEN

Medical Clinics of the State University, Utrecht (The Netherlands)

and

van't Hoff Laboratory, State University, Utrecht (The Netherlands)

INTRODUCTION

In a previous communication[1] we presented evidence of the existence in human gall bladder bile of a complex, containing the total amount of bilirubin and the majority of the cholesterol and bile acids present, thus creating the main constituent of gall bladder bile. The existence of this complex was demonstrated by paper electrophoresis. Simultaneously, several diagrams of the same bile sample were produced and the various constituents demonstrated by means of staining methods*. It appeared that these components migrated with the same velocity as the lipoprotein.

It promised to be interesting to gain further affirmation of the existence of the lipoprotein complex and to carry out more detailed studies in relation to stability problems of gall bladder bile and the formation of gallstones. This communication deals with the results of this investigation.

Demonstration of the existence of the complex by other methods

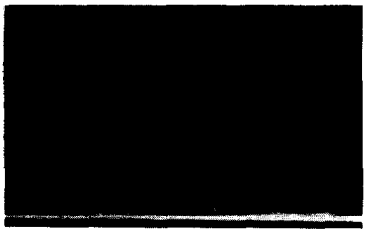
Free electrophoresis. Fig. 1 shows the diagram obtained by free electrophoresis. This gall bladder bile sample contained 9.0 g solutes per 100 g of bile. Dilution 1 : 20 with phosphate buffer of pH 5.8. The sample could not be dialysed against this buffer solution because in this case a strong Tyndall effect appeared.

The electrophoresis was performed in an apparatus according to Tiselius (manufactured by Strübin, Basel). The potential gradient was about 4 V/cm; the electric current 20 mA. The picture was taken on Ferrania P3 film without filters, after 6400 sec and 90 minutes waiting for the diffusion of numerous small salt gradients that were created as a sequence of the omission of previous dialysis.

One single important gradient appears, moving with a velocity much higher than that of albumin. The adherence of bilirubin in the complex cannot be demonstrated by this type of film. It moved, however, with the same velocity as the macromolecule.

* The combination of such a series of stained paperstrips from a single bile sample is further called an "electrochromogram".

References p. 165/166



rising boundary descending boundary

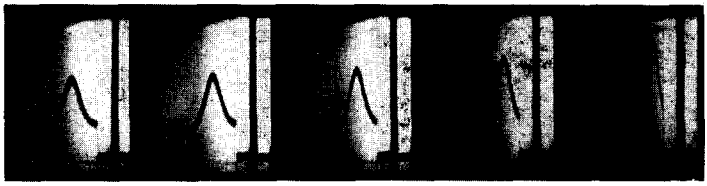
Fig. 1. Diagram of free electrophoresis of gall bladder bile *.

Ultracentrifuge experiments

The sedimentation diagram of normal gall bladder bile always shows one single macromolecular component in a high concentration. Other components are found rarely and in low concentrations.

The diagram was obtained with a Spinco ultracentrifuge model E from a sample of gall bladder bile (No. 4 from Table II). The bile was diluted 10 times with 0.9% NaCl solution. Speed: 59,780 rev/min, temperature *ca.* 17° C, $S_{20} = 1.4 \cdot 10^{-13}$ sec.

All photographs were made at "bar angle" 55° in a "12 mm cell". Nearly all



114 min 95 min after 75 min idem after 45 min after 12 minutes rotating at highest speed

Fig. 2. Sedimentation diagram of gall bladder bile.

the solute sediments as a homogenous compound. Bilirubin is obviously bound to this compound, as was already found in early investigations[13].

The fact that the complex could be demonstrated with the electrochromograms[1] at pH 8.6, with free electrophoresis at pH 5.8, and in the ultracentrifuge experiment, is sufficient proof for its existence.

Determination of the molecular weight of the complex

This was done using the ultracentrifuge. The molecular weight of a pure compound can be calculated with SVEDBERG's formula [2a]

$$M = \frac{RTs}{D\,(1-V\rho)} \qquad (1)$$

where M = molecular weight
R = constant of Boyle's gas law in ergs/degree
T = temp. in °Kelvin at which s and D were measured
s = sedimentation coefficient in sec
D = coefficient of diffusion in cm^2/sec
V = partial specific volume of regarded compound
ρ = density of the solution[3]

* We thank Dr. J. A. NIEMEYER for preparing this preliminary electrophoresis diagram.

References p. 165/166

Formula (1) applies to ideal solutions; this means that the influence of activity coefficients is negligible. The best way to eliminate the remaining concentration dependence of s and D is by extrapolation of the combined value $\frac{s}{D}$ towards concentration = 0[4]. We contented ourselves for the present with calculating M for each bile sample at two concentrations because: (1) there was not sufficient material available for determinations of s and D at more than two concentrations and (2) it cannot be assumed tacitly that the weight of our complex (M) is independent from concentration.

EXPERIMENTAL

(a) Values of s were determined in a Spinco ultracentrifuge model E. This centrifuge is equipped with a Philpot-Svensson optical system. From the diagrams obtained, s can be calculated [2b] from the radial displacement with time of the top of our diagram $\left(\frac{dx}{dc} \text{ versus } x\right)$, with the formula:

$$s = \frac{x_2 - x_1}{4\pi^2 \nu^2 \frac{x_1 + x_2}{2} (t_2 - t_1)} \qquad (2)$$

where: x_1 and x_2 are distances in cm from the axis of rotation to the maximum in concentration gradient at times t_1 and t_2, measured in sec from the beginning of the experiment; and ν = number of revolutions per second.

(b) The determination of D was also carried out in the ultracentrifuge, using a synthetic-boundary cell[5]; in this, a boundary between two solutions may be created. In our case these solutions were: above, solvent (0.9% NaCl), and below, bile, diluted with 0.9% NaCl. The creation of the boundary occurs at *ca.* 5000 rev/min during acceleration of the rotor. Then speed is maintained at 12,590 rev/min. At this speed sedimentation is negligible. From changes in the shape of the $\frac{dc}{dx}$ versus x curve, D can be calculated. For this purpose the curves at different times were drawn in large magnification on graph paper, taking the middle of the line as the right curve. If diffusion is nearly ideal, as under these conditions, then

$$Dt = \frac{d_i^2}{8 V_A^2 V_B^2} \qquad (3)$$

where d_i = distance in cm between the inflection points of the graph.
V_A = enlargement from graph to photographic plate.
V_B = enlargement from photographic plate to cell.
t = time in seconds from beginning of diffusion.

In our method of boundary creation t cannot be known exactly. Therefore we write: $t = t' + \Delta t$, where t' = time (known), passed since the moment of creation of the boundary. So

$$D = \frac{D(t' + \Delta t) - D\Delta t}{t'} = \frac{d_i^2 (t') - d_i^2 (t' = 0)}{8 V_A^2 V_B^2 t'} \qquad (4)$$

References p. 165/166

From 5 photographs d_i^2 is plotted against t', thus giving d_i^2 ($t' = 0$) and D. In earlier experiments with KCl and ovalbumin this method of calculating D proved to be reliable to about 6%. The methods for calculation of s and D, as given above, are valid only for "ideal" curves, *i.e.*, s and D are independent of concentration. If afterwards, as in this case, D and s prove to be dependent on concentration, it is usual to take the concentration of the calculated s or D, as half the initial concentration.

(c) The factor $(1 - \bar{V}\rho)$ was calculated[2c] from curves of density versus concentration. The measurements, however, were not very accurate because of lack of material. As the concentration of organic material, we used the total concentration minus 0.009, because bile always contains *ca.* 1% inorganic material. As the quantity of salts eventually bound to the macromolecules is unknown, we assumed 0.9% to be present as free salts. In this case, salt concentration is not influenced by dilution with 0.9% NaCl. In all cases $(1-\bar{V}\rho)$ proved to be about 0.15.

(d) Temperature at a special moment was known from interpolation between rotor temperature measurements before and after an experiment, and in case of high speeds was diminished by 0.85° C on account of[7] adiabatic cooling of the rotor during acceleration. Values of s and D were corrected to 20° C with[2d] the formulae:

$$S_{20} = \frac{\eta t^0}{\eta_{20}} \quad \text{and} \quad D_{20} = \frac{273 + t^0}{293} \cdot \frac{\eta t^0}{\eta_{20}} \cdot D_t{}^0 \tag{5}$$

where η are (tabulated) viscosities of water.

Results and discussion. The results of measurements in 5 samples of gall bladder bile, obtained by puncture of the gall bladder during laparotomy, are given in Table I.

TABLE I

MOLECULAR WEIGHT MEASUREMENTS

Bile sample from	*Total solids in g%*	*Dilution*	*Half protein conc. at exp. in g%*	$S_{20} \times 10^{13}$	D	*Mol. Weight*
v. d. B.	16.3	10 ×	0.77	1.2		
		25 ×	0.31	1.45	8.15	26.500
O.	16.74	10 ×	0.79	1.4	7.5	28.000
		25 ×	0.40	1.5	7.1	31.500
v. S.	13.3	20 ×	0.31	1.75	7.9	33.000
		36 ×	0.17	1.75	7.8	33.500
v. M.	8.77	10 ×	0.4	1.32	12.5	15.000
		20 ×	0.2	1.44	10.9	20.000
B.	14.45	10 ×	0.68	1.35	9.1	22.000
		20 ×	0.34	1.55	9.1	25.500

All s-values were determined at a speed of 59,780 rev/min. In one case (v. d. B., dilution × 25) M was also determined by the method of ARCHIBALD[8] from a low-speed experiment (20,410 rev/min.). We have already applied this method successfully to other problems. In this case (v. d. B.) we found $M = 24{,}000$.

We estimate the accuracy of the M values at about 10%. Nevertheless in all

References p. 165/166

cases the calculated values of M have a tendency to be higher at the lower concentrations, which indicates that the complex does not disintegrate to any extent after dilution. The differences between bile of various patients are too large to be explained by inaccuracy of the method only. The number of points determined did not allow an accurate extrapolation to infinite dilution, which would make M still somewhat higher than the values given in Table I.

Chemical composition of the complex

From the molecular weight of the complex and the concentration of its various components, the molecular composition of the compound can be calculated. The numerous estimations, however, demand a greater quantity of bile than is mostly obtainable in a single case. In 5 cases, however, samples could be secured that were large enough to allow most of the necessary estimations to be performed. Further data were obtained by analysis of comparable samples of gall bladder bile, and from the literature. All the gall bladder bile samples studied were obtained by puncture of the gall bladder during surgery.

The estimation of the *bile acids* was performed according to the method of KIER[9]. We did not distinguish between cheno-desoxycholic acid and desoxycholic acid. In order to estimate the percentage of bile acids, bound in the complex, filter paper strips were extracted twice with ether and after drying, used for electrophoresis of the bile samples. The band of the complex was cut out and after drying at 37° C, the bile acids were extracted in 10 ml Kier's extraction mixture. The remainder of the filter paper strip was extracted also, in 5 ml Kier's mixture, and bile acid determinations were performed in the ordinary way. It appeared that after exposure to an electric current under the conditions we use for paper electrophoresis[1], cholic acid could not be recovered in appreciable quantities. Thus desoxycholic acid only could be studied. Because this compound is much more liable to form complexes than is cholic acid, it seems probable that this will not seriously influence the conclusions. The *bilirubin content* of the bile was determined by the method of HYMANS VAN DEN BERGH[10].

Cholesterol was determined by our modification of the GRIGAUT method[11]. In the ether extract, we eliminated bilirubin with small quantities of animal charcoal (about 10 mg per sample). Addition of large amounts of charcoal, or filtration, results in losses of cholesterol. The charcoal is eliminated by centrifugation. In this estimation good duplicate results and good recoveries are obtained. The results are more reliable than with methods in which preliminary alkaline hydrolysis is used, and the method is very simple. Furthermore, the product after alkaline hydrolysis gives with the Liebermann-Burchardt reaction a colour with an absorption spectrum markedly different from that obtained with standard solutions prepared with pure cholesterol. Thus the extinctions can not be read accurately from a calibration curve.

The cholesterol in gall bladder bile is partly bound in the complex. In our first communication[1] we gave a mean value of 79% of the total cholesterol as bound in the complex. This value was used for the calculations of the molecular constitution of the complex.

The results of the various estimations, together with data from the literature, are given in Table II.

From these data the order of magnitude of the molecular constitution of the complex was calculated. For the lipid component the data of POLONOVSKI AND

References p. 165/166

TABLE II

Case	*Diagnosis*	*Total solutes in g%*	*Organic substance in g%*	*Molecular weight of complex*	*Bilirubin mg%*	*Cholesterol mg%*	*Cholic acid g%*	*Desoxycholic acid*	
								total g%	*bound in % of total*
1. v. M.	normal	8.77	7.64	18.000	53.3	375	1.78	4.0	70%
2. B.	normal	14.45	13.40	23.500	121.8	665	1.65	7.2	69%
3. A.	normal	9.24	8.19	—	104.3	330	1.24	5.93	81%
4. O.	normal	14.16	12.44	29.000	394.9	—	—	—	—
5. v. S.	hemolytic jaundice	13.29	11.87	33.000	846.9	689	2.50	5.50	47%
6. v. d. B.	gall stones	16.3	15.3	26.500	95.2	594	2.3	8.83	76%
7. R.	stones	4.11	3.60		9.8	254	0.01	2.48	78%
8. J.	stones	17.11	15.38		257.5	567	2.06	9.26	89%
9. v. d. H.	stones	4.95	4.37		28.9	139	0.26	2.99	84%
10. N.	stones	8.87	8.12	mean	174.9	937	0.05	5.07	90%
11. T.	stones	7.76	7.23	26.000	57.4	246	1.22	3.52	85%
12. M.	stones	8.55	7.73		88.0	466	0.71	- -	78%
13. Ar.	stones	9.02	8.36		107	372	0.05	5.81	63%
14. Mu.	stones *	2.45	2.19		0.16	20	0.0	0.35	- -
POLONOWSKY [14]		18.0			140	430		11.5	lecithin 3.5 g
COLP *et al.* [15]								7.7–9.8	
WALTERS AND SNELL [16]					80–135	160–260			
LICHTMAN [17]						5–600			
REINHOLD [18]						260–540			

* colourless bile.

BOURRILLIN[12] were used. These authors found that there was a constant relationship between phosphorus, choline and fatty acids in bile and from these and other findings they concluded that the lipids in bile are exclusively present as lecithins. Their concentration was found to be about 15% of the total solids of gall bladder bile. The data for the approximate molecular constitution of the complex are given in Table III. Inorganic salts and cholic acid, as well as the traces of carbohydrates, had to be left out of consideration.

The first two bile samples in Table III were taken from normal individuals. The other samples came from gall bladders containing gallstones. The last sample was from a patient suffering from a hemolytic disease and the bile was collected during a resection of the spleen *. It shows clearly that the complex is certainly not a compound of constant composition. Fluctuations in a number of bilirubin or cholesterol molecules, bound in the complex, are dependent in the first place on the hepatic excretion of these substances. At most 9 molecules of bilirubin and 7 molecules of cholesterol were found in one molecule of the complex. The fact that in the case v.S. (last of the series from Table III) the complex was hardly able to take up any azo-

TABLE III

Bile sample from	*Mol. weight complex*	*Molecular composition of the complex*			
		Mean no. of mol./mol. complex			
		Bilirubin	*Cholesterol*	*Desoxycholic acid*	*Lecithin*
v. M.	18.000	0.3	3.0	27.4	6.5
B.	23.500	0.7	3.6	33.3	9.5
v. d. B.	26.500	0.5	2.7	42.3	7.8
R.		0.2	4.0	43.0	6.9
J.		0.1	2.4	45.2	7.0
v. d. H.	mean	0.4	2.1	46.2	6.9
N.	26.000	1.3	6.8	40.9	6.0
T.		0.6	2.4	36.4	7.1
A.		1.0	3.3	39.7	7.4
v. S.	33.000	9.4	6.6	21.9	13.5
Mean values (without v. S.)		0.57	3.4	39.8	7.2

carmin during staining of the proteins, suggests that the point of saturation for bilirubin is reached. Under normal conditions some molecules of the complex must be free of bilirubin while others bear one or more molecules of this substance.

This finding is supported by the fact that in most instances the lipoprotein band in the paper electrophoresis diagram appears to be inhomogeneous. It often contains a zone of stronger yellow colour and a slightly lower electrophoretic mobility, which takes an azocarmin stain with rather more difficulty than the pale zone that runs ahead of it. The higher the bilirubin concentration of a bile sample, the greater is the part of the complex band that is taken by the stronger coloured product.

* We thank Dr. M. KLINKENBERGH and Dr. A. SCHAEPKENS VAN RIEMPST for their cooperation in collecting numerous gallbladder bile samples.

References p. 165/166

The major part of the complex is always created by desoxycholic acid molecules. The role played by the "choleic acid principle" in the construction of the macromolecule is further investigated. Still, we consider the name "lipoprotein complex" appropriate because with paper electrophoresis the complex has never been found in the absence of the lipoprotein compound. With the constituents given in Table III, the weight of the complex molecule is covered for 85 to 95%. The remainder, probably consisting of a protein component (and eventually small quantities of some other substances) must therefore have a molecular weight of the order of 4,000 and certainly not exceeding about 10,000. *The lipoprotein must thus be built up from one (or more) molecules of a polypeptide together with about 7 molecules of lecithin.* Affirmation by isolation and further study of this polypeptide provide subject for further investigation.

Properties of the complex

Its *stability* proved to be very high. Sterile gall bladder bile, taken during operation and kept in the refrigerator in closed vessels at a temperature of *ca.* 4° C for one month gives the same electrochromogram as the fresh sample. Dilution with 0.9% NaCl solution and reconcentration did not change the pattern.

Gall bladder bile proved to be amazingly resistant against bacterial contamination. Although not kept under sterile conditions and opened often for pipetting with unsterilised pipets, 4 out of 6 bile samples were sterile after one to 3 months. The other 2 showed growth of *Escherichia coli*. The bili-lipoprotein complex in the infected samples kept as well as in the sterile samples.

Oxidation of bilirubin bound in the complex is very slow. From 3 samples (gall bladder bile, nos. 3, 5 and 8 from Table II) that had been standing in contact with air, mainly in the refrigerator at 4° C, 45, 70 and 34% of bilirubin, respectively, could be recovered after 50 to 55 days, with the Hymans van den Bergh reaction.

Extraction of the complex with ether yields about 30% of the total cholesterol present in the gall bladder bile sample. With chloroform, about 60% of the total cholesterol could be extracted. Less then 10% of the phosphatides could be extracted with ether. They are more firmly bound than cholesterol. However, the mixture abs. alcohol-ether-acetone 1 : 1 : 1, (extraction mixture of KIER), dissolves the complex completely; the remaining 10% of the total solids precipitates. The precipitate consists of a protein with a much smaller electrophoretic mobility than the complex, and of bilirubin. The precipitate, after being washed with fresh extraction mixture, contains no appreciable amounts of cholesterol, lipids or bile acids.

Precipitation is best carried out with ammonium sulfate. The complex precipitates quantitatively in a solution half saturated with ammonium sulfate. Some precipitation occurs even in solutions one quarter saturated with the salt. Some decomposition of the precipitate is evident. After being redissolved in barbitone buffer of pH 8.6 it shows a broad band with paper electrophoresis. The largest part of the complex shows a much smaller electrophoretic mobility than the native complex, suggesting that part of its electrically charged components have been split off.

Staining properties. Comparison of the azocarmin, bromphenol blue and amido black stains was definitely in favour of the azocarmin. There is a reverse relationship between the staining of the complex and its bilirubin content. Probably the bilirubin occupies the same groups at the complex molecule as the azocarmin.

References p. 165/166

Occurrence of the complex

This was studied in 360 electrochromograms[1], from the following groups of cases.

1. Normal gall bladder bile, obtained during surgery for other than gall bladder disease, 7 cases.

2. Gall bladder bile showing good concentration with cholecystography from cases with uncomplicated stone disease, 23 cases.

3. Gall bladder bile from cases with stones and obvious signs of chronic inflammation of the gall bladder, obtained exclusively by direct puncture during surgery, 17 cases.

4. Colourless bile from obstructed gall bladders, 5 cases.

5. Gall bladder bile obtained fresh from various animals 12 cases.

6. Bile taken from the hepatic duct by puncture, 11 cases.

7. Fistula bile samples from 54 patients, in most cases with daily follow-up. In total 285 diagrams.

Results. In normal gall bladder bile, the lipoprotein complex was always present in high concentration. The other biliary proteins, moving with lower velocities and called P(rotein) 2 and P3, were found in much lower concentration. P2 was not detectable in 2 out of 7 cases and present in traces in 3 more cases. P3 was only found in traces in 2 cases.

In uncomplicated gallstone disease, the complex was present in normal amounts in 7 out of 23 cases studied. In all the other cases it was found in lower concentration. Both the other proteins were present, generally in higher concentrations than in the normal bile samples.

In the 17 gall bladder bile samples from cases with apparent inflammation of the gall bladder, normal amounts of the complex were never encountered. In 8 cases, no complex could be found at all. In the 9 other cases, only traces or very low concentrations were found. Both P2 and P3 were present in highly elevated quantities. In the 5 cases of colourless bile, no complex was found. From these findings it is clear that inflammation runs parallel with depression of the bili-lipoprotein content of gall bladder bile, not only absolutely but also in comparison with other constituents.

As was previously noted[1], the lipoprotein is absent in most of the specimen of fistula bile. In the material obtained since our first communication we found that the longer a bile fistula exists and the less the degree of inflammation, the more is the chance that the complex will appear, mostly in traces, but in several cases in appreciable amounts. In 285 diagrams of fistula bile the lipoprotein was not detectable in 2/3 of the cases. In follow-up studies of 24 cases with bile fistulas existing longer than 5 days, in 13 instances the lipoprotein appeared after 3 to 15 days postoperatively. Sometimes it disappeared again. It seldom reached levels comparable with gall bladder bile, even after intense artificial concentration.

In the 11 cases in which bile could be obtained directly by puncture of the hepatic duct, lipoprotein was found in small amounts in 3 cases and in traces in 3 more cases.

A preliminary study in animals disclosed that in the gall bladder bile of dogs and cats conditions exist that resemble closely those found in the human being. Cows and pigs had a much lower concentration of lipoprotein. In the gall bladder bile of guinea pigs it could not be detected. Rabbits had very little. The significance of these findings for comparative pathology is under investigation.

References p. 165/166

Place of formation of the complex

The fact that with paper electrophoresis the complex is often undetectable in liver bile and abundantly present in all samples of non-inflammatory gall bladder bile suggests that it is produced by the gall bladder mucosa. In that case also the walls of the large bile passages would need to be able to produce the complex under certain conditions. The cells of the gall bladder mucosa contain a well developed Golgi reticulum, indicating some excretory function, as yet unknown. These facts, together with the concentration ratios for lipids, choline, etc. in comparison with those of bilirubin, cholesterol and proteins, point in the direction of production of lipoprotein by the lining of the gall bladder and eventually bile passages. Strict evidence, however, is not yet obtained. The question is of major importance in connection with the significance of the lipoprotein for gallstone formation and must be further investigated.

SUMMARY

The solutes of human gall bladder bile appear to exist mainly in the form of a complex macromolecule, formed around a nucleus of lipoprotein. The existence of this macromolecule was demonstrated by paper electrophoresis[1], free electrophoresis and ultracentrifuge experiments. The molecular weight of the compound was found to be of the magnitude of 26,000. Evidence was obtained that the complex has no completely constant composition. The main molecular constitution per molecule of the complex was calculated as: 0.57 mol. bilirubin, 3.4 mol. cholesterol, 39.8 mol. desoxycholic acid and 7.3 mol. lecithins, probably grouped around one or more molecules of a polypeptide with a molecular weight below 10,000. As this complex is the main macromolecular constituent of gall bladder bile and contains all the bilirubin present, and about 80% of the cholesterol, it must be involved in stabilising the gall bladder bile and thus be related to gallstone formation. The concentration of the complex was found to be lower, the more inflammatory signs were present in the gall bladder. The properties of the complex, such as stability, resistance to extraction procedures, precipitation and staining properties, were studied. Finally, the occurrence of the bili-lipoprotein was studied in 360 bile samples, 64 from gall bladder bile, all obtained by puncture of the gall bladder during surgery, and 296 samples of fistula bile and samples obtained by puncture of the hepatic duct during surgery. The lipoprotein complex, always abundantly present in gall bladder bile if no inflammation of the gall bladder exists, was absent in 2/3 of the fistula bile samples. In fistula bile it is never found in concentrations as great as in gall bladder bile. Artificial concentration of fistula bile that contains an appreciable amount of the complex seldom gave a product that was equal to or closely resembling gall bladder bile. These and other facts suggest production of lipoprotein within the gall bladder, and in minor quantities under certain conditions by the walls of the bile passages.

RÉSUMÉ

Les solutés de la bile de vésicule biliaire humaine semblent exister surtout sous forme d'une macromolécule complexe, formée autour d'un noyau lipoprotéinique. L'existence de cette macromolécule a été démontrée par électrophorèse sur papier[1],

References p. 165/166

par électrophorèse libre et par des expériences d'ultracentrifugation. Nous avons trouvé que le poids moléculaire du composé était de l'ordre de 26,000. Certains faits font penser que la composition du complexe n'est pas tout-à-fait constante. La constitution moléculaire a été calculée comme suit pour une molécule de complexe: 0.57 mols de bilirubine, 3.4 mols de cholésterol, 39.8 mols d'acide désoxycholique et 7.3 mols de lécithine, groupés probablement autour d'une ou de plusieurs molécules d'un polypeptide, à poids moléculaire inférieur à 10,000. Comme ce complexe est le constituant macromoléculaire principal de la bile de vésicule biliaire et qu'il contient toute la bilirubine présente et environ 80% du cholestérol, il doit jouer un rôle dans la stabilisation de la bile de vésicule biliaire et être lié ainsi à la formation des calculs biliaires. La concentration du complexe était d'autant plus basse que les signes d'une inflammation de la vésicule étaient plus nombreux. Les propriétés du complexe telles que stabilité, résistance contre les procédés d'extraction, la précipitation et les propriétés de teinture ont été étudiées. Finalement l'occurrence de la bili-lipoprotéine a été étudiée dans 360 échantillons de bile, 64 de bile de vésicule biliaire, obtenus par ponction de la vésicule biliaire pendant une opération, et 296 échantillons de bile de fistule et des échantillons obtenus par ponction du canal hépatique pendant une opération. Le complexe lipoprotéinique qui est toujours abondamment présent dans la bile de vésicule biliaire pourvu que cette vésicule ne soit pas inflammée, était absent dans 2/3 des échantillons de bile de fistule. On ne le trouve jamais dans la bile de fistule dans des concentrations aussi élevées que dans la bile de vésicule biliaire. Des concentrations artificielles de bile de fistule qui contenaient une quantité appréciable de complexe n'ont donné que rarement un produit égal ou très ressemblant à la bile de vésicule biliaire. Ces faits et d'autres encore nous suggèrent l'idée que la lipoprotéine est formée à l'intérieur de la vésicule biliaire et, en quantités inférieures et sous certaines conditions, par les parois des canaux biliaires.

ZUSAMMENFASSUNG

Die gelösten Bestandteile von menschlicher Gallenblasengalle scheinen hauptsächlich in Form eines komplexen Makromoleküls zu bestehen, das sich um einen Lipoproteinkern gebildet hat. Die Existenz dieses Makromoleküls wurde durch Papierelektrophorese[1], freie Elektrophorese- und Ultrazentrifugenversuche bewiesen. Das Molekulargewicht der Verbindung war von der Grössenordnung 26,000. Gewisse Befunde weisen darauf hin, dass die Zusammensetzung des Komplexes nicht ganz konstant ist. Die Grundzusammensetzung wurde pro Molekül wie folgt berechnet: 0.57 Mol Bilirubin, 3.4 Mol Chesterol, 39.8 Mol Desoxycholsäure und 7.3 Mol Lecithin, wahrscheinlich gruppiert um ein oder mehrere Moleküle eines Polypeptides dessen Molekulargewicht niedriger ist als 10,000. Da dieser Komplex der hochmolekulare Hauptbestandteil der Gallenblasengalle ist und das gesammte Bilirubin und ungefähr 80% des Cholesterols enthält, muss er bei der Stabilisierung der Gallenblasengalle eine Rolle spielen und so mit der Gallensteinbildung zusammenhängen. Die Konzentration des Komplexes war umso niedriger, als mehr Anzeichen einer Entzündung in der Gallenblase vorhanden waren. Die Eigenschaften des Komplexes wie Stabilität, Widerstand gegen Extraktionsverfahren, Präzipitation und Färbeeigenschaften wurden untersucht. Schliesslich wurde das Vorkommen des Bili-Lipoproteins untersucht, in 360 Gallenproben, 64 aus Gallenblasengalle, alle durch Punktion der Gallenblase

References p. 165/166

während einer Operation erhalten, und 296 Proben von Fistelgalle und Proben welche durch Punktion des Leberkanals während einer Operation erhalten waren. Der Lipoproteinkomplex, welcher immer reichlich in der Gallenblase vorhanden ist, wenn keine Entzündung der Gallenblase besteht, fehlte in 2/3 der Fistelgallenproben. In Fistelgalle wird er niemals in so hoher Konzentration gefunden, wie in Gallenblasengalle. Künstliche Konzentrate von Fistelgalle, die eine merkbare Menge Komplex enthielten, ergaben niemals ein Produkt, das der Gallenblasengalle gleich oder sehr ähnlich war. Aus diesen und anderen Tatsachen kann man schliessen, dass das Lipoprotein wahrscheinlich innerhalb der Gallenblase und in kleineren Mengen unter gewissen Bedingungen durch die Wände der Gallenwege gebildet wird.

РЕЗЮМЕ

Разстворенное содержание человеческой желчи из желчнаго пузыря находится вероятно в большинстве случаев в виде комплексных молекул групированных вокруг липопротеиновых ядер. Существование этих макромолекул было наглядно доказано бумажным електрофорезисом, прямым електрофорезисом и методом ультросентрифугации, Молекулярный вес этого вещества был найден в порядке 26.000. Было доказано, что этот комплекс не имеет определеннаго состава. Средний молекулярный состав разчитанный на молекулу комплекса был: 0,57 мол. билирубина, 3,4 мол. холестерола, 39,8 мол. дезоксихолиновой кислоты и 7,3 мол. лицина, сгрупированные вокруг одной или больше молекул полипептида с молекулярным весом ниже 10,000. Так как этот комплекс является главным макромолекулярным составляемым желчи из желчнаго позыря и содержит весь присутствующий билирубин и приблизительно 80 % холестерола, он должен играть роль в стабилизации желчи желчнаго пузыря и таким образом влиять на образование желчных камней. Чем более ясны были признаки восполения желчнаго пузыря, тем ниже была концентрация комплекса. Следующии качества комплекса были изучены: устойчивость, сопротивление к вытяжке, к осаждениюо и окраска. Всего было исследовано 360 образцов желчи на присутствие били-липопротеина из которых 64 из желчных пузырей полученных пунктированием этих пузырей во время операции и 296 образцов желчи из желчнаго прохода, также полученные пункцией печечнаго прохода во время операции. В то время как липопротеиновый комплекс обильно присутствовал в желче из невоспаленных желчных позырей, этот комплекс отсутствовал в 2/3 образцов желчи из желчнаго прохода. В желчи из желчнаго прохода он не был ни разу найден в таких больших концентрациях как он находился в желчи из желчнано пузыря. Искуственное сгущение желчи из желчнаго прохода, содержащий довольно значительное количество комплекса, ни разу не дал вещества равнаго или похожего на желч из жечлнаго пузыря.

Эти и другия данныя дают возможность предположить производство били-липопротеина внутри желчнаго пузыря и в меньшей степени, при особых обстоятельствах, в стенках желчнаго прохода.

REFERENCES

1 J. C. M. VERSCHURE, *Clin. Chim. Acta*, *1* (1956) 38.
2a THE SVEDBERG AND K. O. PEDERSEN, *Die Ultrazentrifuge*, Th. Steinkopff, Dresden, 1940; b *ibid.*, p. 244; c *ibid.*, p. 52; d *ibid.*, p. 244, 247.
3 G. L. HOOYMAN, *Thesis*, Leiden, 1955, p. 15.
4 R. L. BALDWIN AND A. G. OGSTROM, *Trans. Faraday Soc.*, *50* (1954) 749.
5 E. G. PICKELS, W. F. HARRINGTON AND H. K. SCHACHMAN, *Proc. Natl. Acad. Sci. U.S.*, *38* (1952) 943.
6 O. LAMM, *Nova Acta Regiae Soc. Sci. Upsaliensis*, *10*, No. 6 (1937) 33.
7 D. F. WAUGH AND D. A. YPHARTIS, *Rev. Sci. Instr.*, *23* (1952) 609.
8 W. J. ARCHIBALD, *J. Phys. & Colloid Chem.*, *51* (1947) 1204.
9 L. C. KIER, *J. Lab. Clin. Med.*, *40* (1952) 755.

10 A. A. HYMANS VAN DEN BERGH, *Brit. Med. J.*, *1* (1934) 1157.
11 A. GRIGAUT, *Compt. rend. soc. biol.*, *71* (1911) 513.
12 M. POLONOVSKI AND R. BOURRILLON, *Bull. soc. chim. biol.*, *34* (1952) 712.
13 K. O. PEDERSEN AND J. WALDENSTROM, *Hoppe-Seyler's Z. physiol. Chem.*, *245* (1937) 152.
14 M. POLONOVSKI AND R. BOURRILLON, *Bull. soc. chim. biol.*, *34* (1952) 703.
15 R. COLP AND H. DOUBILET, *Arch. Surg.*, *33* (1936) 913.
16 W. WALTERS AND A. M. SNELL, *Diseases of the Gallbladder and Bile Ducts*, Saunders, Philadelphia, 1940.
17 S. S. LICHTMAN, *Diseases of the Liver, Gallbladder and Bile Ducts*, Lea & Febiger, Philadelphia, 1942.
18 J. G. REINHOLD, L. K. FERGUSON AND A. HUNSBERGER, JR., *J. Clin. Invest.*, *16* (1937) 367.

Received January 8th, 1956