ON THE BEHAVIOR OF LECITHIN WITH BILE SALTS, AND THE OCCURRENCE OF LECITHIN IN BILE.

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Received June 4, 1908.

In several handbooks of physiological chemistry lecithin is referred to as one of the ordinary bile constituents, although the amounts present are assumed to be very small.[1] On the other hand, Thudichum states distinctly that ox bile does not contain lecithin, although a phosphatide of other nature appears to be present.[2] It must be granted that the evidence for the occurrence of lecithin in bile is not very convincing, as its presence is usually inferred from the phosphorus content of alcohol-ether extracts, and in the light of many recent investigations conclusions from such facts have no great weight. The most important direct studies of the subject are doubtless those of Hammarsten, which are recorded in several recent works.[3] While leaving the question of the universal occurrence of lecithim in bile still open Hammarsten concludes from his several investigations that it is doubtless present in the bile of the polar bear, which was the subject of numerous experiments.

We have recently undertaken a further examination of the subject, and have made experiments with ox bile and also with human bile. The results of the work with the ox bile will be given first. At the outset we hoped to effect a separation of the phosphorus-holding complex from the great mass of salts by variations in the methods of extraction, but, as the sequel shows, we were practically unsuccessful in this. We began by evaporating considerable quantities of fresh bile nearly to dryness, at a comparatively low temperature on the water-bath, which consumed several days for two portions of two liters each. The two residues, which were brought down to the well-known pasty condition, were treated then in different ways.

One residue was transferred to a liter flask and extracted under a reflux condenser with a mixture of alcohol and ether, with the expectation of leaving the larger part of the bile salts undissolved, and of bringing any lecithin present into solution. This operation was repeated a number of times on the same residue and the various extracts, which were easily poured off, were united. This mixture was then treated with a very considerable excess of ether which threw down some bile salt dissolved. The larger volume of supernatant ether was distilled and the small residue left was tested for phosphorus and nitrogen. This residue contained some fat-like matter but did not give even a faint

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[1] See the well-known handbooks of Hoppe-Seyler and Hammarsten.

[2] Die chemische Konstitution des Gehirns des Menschen und der Thiere.

[3] *Z. physiol. Chem.*, 32, 435; 36, 525; 43, 109. Also, *Ergebnisse der Physiologie*, 4th year, p. 1.

reaction for either of the elements just mentioned. The crystalline precipitate formed by the addition of ether contained phosphorus. The larger amount of residue in the original extraction flask was reserved to be worked up as explained below.

A second mass resulting from the evaporation of two liters of bile was extracted repeatedly with considerable volumes of pure acetone, in which fats and cholesterol are soluble, but lecithin and dry bile salts practically not. It was hoped to secure in this way a product from which a subsequent separation of lecithin might prove easier. The residue left after the acetone extraction, was accordingly treated as in the other case above described, with an excess of ether and enough alcohol to soften the mass and then boiled under a reflux condenser a number of days. As the mass settled quickly in the boiling flask, it was possible to secure a clear ether-alcohol solution by pouring. All the solutions secured in this way were united and treated as before with an excess of ether for precipitation of dissolved salts. The experience in this case was the same as in the other. The supernatant ether when evaporated left no residue containing phosphorus or nitrogen, while the crystalline precipitate thrown out gave sharp tests for both. Nitrogen, of course, would naturally be present from the bile salts.

As these trials failed to give any indication of free lecithin in the ether solutions, another experiment was made with bile not evaporated. A volume of two liters of this bile was repeatedly shaken with fresh portions of ether and the supernatant layers decanted and united. From this mixture, the greater part of the ether was distilled off and the residue was tested for phosphorus with entirely negative results. As ether does not extract lecithin from its aqueous emulsions unless certain amounts of salts are present,[1] an excess of sodium chloride was added to the bile mass, which was then treated with more ether and shaken thoroughly. As the addition of salt favors the solution of lecithin, it was expected that this same general action would follow here. But no such result was reached, from which it follows that the union between the bile salt and the phosphorus-holding complex must be a very stable one. The experiment was further made by rubbing up enough salt with the bile to secure a pasty mass, which was then thoroughly shaken with ether. The results here were also negative, which strengthens the conclusion regarding the stability of the combination or mixture. In the various experiments of Hammarsten referred to,[2] the presence of lecithin was inferred from the detection of certain products of decomposition rather than from any separated lecithin.

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[1] Long and Gephart, THIS JOURNAL, **30**, 895.

[2] *Loc. cit.*

The fact that bile salts have a marked solvent action on lecithin has been long known,[1] but is usually overlooked in the literature. In addition to this solvent action there is probably a more or less stable combination, as the above, and other experiments to be detailed, suggested. It is stated by Hammarsten that the salts separated from bile always contain phosphorus, and this we have always found in our own experiments. After trying to recognize lecithin in the solutions freed as well as possible from these salts, the principal residues left were worked up to prepare quantities of the mixed salts. These residues were dissolved in alcohol and filtered to separate mucin substances. The clear solutions were mixed and agitated thoroughly with purified animal charcoal until all color was removed. The filtered alcoholic solutions were then precipitated by a considerable excess of ether, and the gummy precipitate which formed at first was thoroughly washed with more ether until it was clear white. This mass when spread out on a glass plate to dry in a current of warm air gradually became crystalline.

The crystalline product so obtained was found to consist essentially of sodium salts of the two principal acids, and on analysis gave these data:

$[\alpha]_D^{20} = 20.93°$ for $C = 5.000$
$S = 3.70$ per cent.
$P = 0.29$ per cent.
$N = 2.64$ per cent.

The rather large amount of phosphorus present is remarkable, and if calculated as lecithin would indicate a constant of 7.5 per cent. of this compound if we assume it to exist as a monamino-monophospthide, or typical lecithin. The sulphur content, as should be expected, suggests a mixture of salts with the taurocholate in excess. Sodium taurocholate contains 5.95 per cent. of sulphur. The nitrogen appears a little low, as the content in sodium glycocholate is 2.87 per cent. and in the taurocholate 2.61 per cent. But this discrepancy disappears if we assume that 7.5 per cent. of the substance is lecithin with a nitrogen content of about 1.74 per cent. Not much is shown by the optical rotation, as the two bile salts are active and in about the same degree. According to Ulpiana,[2] lecithin, also, is active with $(\alpha)_D = +11.3° - 11.4°$, which is lower than the rotation of the bile salts. The three specific rotations concerned are then:

Lecithin, $[\alpha]_D$	+11.3°
Sodium glycocholate[3]	+20.8°
Sodium taurocholate[3]	+21.5°

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[1] See Hoppe-Seyler's *Physiologische Chemie.*

[2] *Jahresbericht* (Maly), 1902, p. 63.

[3] Landolt-Long, *Optical Rotation*, p. 719.

The accurate determination of the rotation of solutions of our mixture is attended with some difficulty as the salt dissolves in water with slight color, and the solution is somewhat viscous. On filling the 200 mm. tube and allowing it to stand at a uniform temperature for several hours it was, however, found possible to secure a series of pretty close readings which we consider accurate enough for the purpose of the following table. If we calculate all the phosphorus and a corresponding part of the nitrogen as belonging to the ordinary lecithin, the sulphur content shows that the residue must be made up almost exactly of two parts of taurocholate and one part of glycocholate. The relations on this basis are shown as follows:

	Theoretical composition.				
	Mixture. Per cent.	N. Per cent.	S. Per cent.	P. Per cent.	Part of rotation.
Lecithin	7.5	0.13	0.00	0.29	0.85
Taurocholate	62.0	1.61	3.70	0.00	13.33
Glycocholate	30.5	0.88	0.00	0.00	6.34
Sum	100.0	2.62	3.70	0.29	20.52
Found	.....	2.64	3.70	0.29	20.93

This method of calculation gives a consistent interpretation of the results as found by analysis and other tests. In the calculation of the rotations, it is assumed that the fractions due to the possible lecithin content and the salts are simply additive. Various phenomena suggest that the union of the salts and lecithin is a physical one, although of a peculiar kind. It has been found by experiment that the salts will dissolve a very large additional amount of lecithin; in fact 5 grams of the salts in 50 or 100 cc. of water will dissolve as much as 4.1 grams of lecithin in the course of a few hours to produce a clear liquid although somewhat colored. In dilute solution the action is slower. The total amount of lecithin which may be dissolved in this way remains about 80 per cent. of the weight of the bile salts. The action is much hastened by the addition of certain metallic salts of which barium chloride is a good illustration. With this substance present the mixture of the bile salts and lecithin clears up almost immediately, but the amount which may be dissolved is not increased. If an excess of the lecithin is taken, this forms an emulsion which separates on standing as it would with the barium chloride alone. In other words, barium chloride will precipitate the lecithin in the usual way as already shown by us,[1] and if the bile salts are present in sufficient amount the precipitate will go completely into solution. But if the lecithin amounts to more than 80 per cent. of the weight of the bile salts, the excess of lecithin settles out.

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[1] THIS JOURNAL, **30**, 895.

This quantitative relation would seem to suggest a chemical union but that this is probably not the case is shown by a further fact. It has been found possible to effect a separation of these artificial mixtures almost completely by simple precipitation with acetone which was done as follows: 10 grams of bile salts and 8 grams of lecithin were dissolved to make a clear solution with 250 cc. of water. A portion of this was precipitated with acetone using a considerable excess. While the dry bile salts are insoluble in acetone, practically no precipitate of these substances forms in presence of sufficient water. The precipitate which came down in this case was light colored and had the appearance and behavior of the usual precipitates which are separated from emulsions by aid of acetone. It was entirely free from the peculiar taste characteristic of the bile salts and contained no sulphur. On the other hand, the acetone filtrate on examination for phosphorus was found to contain more than the amount due to the original bile salts with their assumed lecithin content. This excess should be expected as acetone does not precipitate the emulsion completely, as shown in the former paper.[1] The larger part of the lecithin was, however, contained in the precipitate.

Another experiment was carried out to show the completeness of precipitation of mixtures of bile salts and lecithin by use of ether. The salts employed in this case contained more phosphorus and less nitrogen than the sample analyzed before and described above, *viz.*,—0.38 per cent. of phosphorus and 2.43 per cent. of nitrogen. A mixture containing 5 grams of this salt and 3.95 grams of lecithin, both calculated as anhydrous, was made up in water and then evaporated to dryness. The residue was taken up with alcohol and precipitated by ether in excess. This precipitate was a gummy mass which was dried slowly to the anhydrous condition and then tested. It was found to contain P = 1.58 per cent. and N = 2.24 per cent. These figures show that the lecithin is not fully precipitated as such with the bile salts as the following considerations will make plain.

Theory.		Found in ppt.
Phosphorus in 5.000 grams bile salt	0.019	....
Phosphorus in 3.95 grams lecithin	0.151	....
	0.170	....
Per cent. phosphorus in mixture	1.89	1.58
Nitrogen in 5.000 grams bile salt	0.1215	....
Nitrogen in 3.95 grams lecithin	0.0766	....
	0.1981	....
Per cent. nitrogen in mixture	2.21	2.24

As the per cent. of nitrogen in the bile salts is greater normally than

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[1] THIS JOURNAL, 30, 881.

that in the lecithin, a slightly deficient precipitation of the latter would make the nitrogen appear higher in the precipitate. On the other hand, as the phosphorus content of the mixture is due largely to the lecithin added, an incomplete precipitation of this would give a product showing necessarily lower phosphorus, and these are the conditions presented in the figures above. A little calculation shows that about one-third of the lecithin has not been carried down in the precipitate. These figures show pretty clearly the extent to which bile salts and lecithin may be carried down together in the manner usually employed in the examination of bile. It is evident that a small amount of lecithin may not be separated in this way as the bile salts are able to hold a very considerable weight of lecithin in the precipitation. A small lecithin content in the bile would, therefore, always be found in the salt precipitate formed by ether.

Reference was made above to the action of barium chloride in aiding the solution of lecithin by the bile salts. A somewhat similar action was noticed with many other inorganic salts. It will be recalled that aqueous emulsions of lecithin are precipitated by a great number of salt solutions[1] and we now find that most of them assist the bile salts in dissolving lecithin. In consequence of this when solutions of inorganic salts are added to the clear solutions of lecithin and bile salts no turbidity follows as with the thin lecithin emulsions above. There appear, however, to be a few exceptions, as the following table will show. The solution of bile salts used was of five per cent. strength, and the lecithin added amounted to about four per cent. In each case portions of 5 cc. of this solution were mixed with an equal volume of the various salt solutions, usually of normal strength. When the solubility was below this, saturated solutions were used. In the table + indicates the formation of a precipitate, and — not. The behavior with the bile salt solution alone is given for comparison, and the action of a few acids is added. It appears that the mixture is precipitated in those cases when the bile salt above is decomposed, with the separation or precipitation of its acid.

	Bile salt solution.	Bile salt plus lecithin.		Bile salt solution.	Bile salt plus lecithin.		Bile salt solution.	Bile salt plus lecithin.
NaCl	—	—	$CaCl_2$	—	—	$Cu(NO_3)_2$	—	—
KCl	—	—	$SrCl_2$	—	—	$HgCl_2$	—	—
Na_2SO_4	—	—	$BaCl_2$	—	—	$CdCl_2$	—	—
K_2SO_4	—	—	$Ba(NO_3)_2$	—	—	Tl_2SO_4	—	—
$(NH_4)_2SO_4$	—	—	$MgSO_4$	—	—	$Pb(NO_3)_2$	+	+
$K_2C_2O_4$	—	—	$AlCl_3$	+	+	$Hg(NO_3)_2$	+	+
KSCN	—	—	$FeCl_3$	+	+	HCl	+	+
$NaNO_3$	—	—	$KAl(SO_4)_2$	+	+	HNO_3	+	+
KNO_3	—	—	$Al_2(SO_4)_3$	+	+	H_2SO_4	+	+
NH_4NO_3	—	—						

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[1] THIS JOURNAL, 30, 898.

The experiments recorded above were all made with salts from ox bile. We had at our disposal a small quantity of human bile with which a number of analogous tests were made. The bile salts thrown down here contained, likewise, a phosphorus compound, but this, as in the other case, could not be separated by any solvent at our disposal, or by any method of precipitation. The amount of bile available did not permit us to make an exact determination of the phosphorus present.

Optical Rotation.—When it was pointed out some years ago by Ulpiana[1] that the ordinary lecithins possess optical rotatory power, the discovery occasioned some surprise as the presence of asymmetric carbon in the complex was not suspected. We have attempted to throw further light on the question by finding the rotation of lecithin in a solution of bile salt which is much less highly colored than the solutions in alcohol or ether. It must be admitted, however, that the value of such determination could not be final because of the behavior of the bile salts themselves.

The solution used contained 5 grams of bile salts and 4 grams of lecithin in 100 cc. and in the 100 mm. tube gave a rotation of 1.36°. Under the same conditions, the bile salts alone rotate 1.05°, leaving 0.31° as the effect of the 4 grams of lecithin in 100 cc. If we may assume that the action is merely an additive one and that neither substance affects the rotation of the other, this would give for the lecithin complex

$$[\alpha]_D = +7.75°.$$

The value reported by Ulpiana was considerably higher than this, but it is possible that the different lecithins exhibit a somewhat different behavior in this respect.

Summary.

1. Bile salts, as ordinarily obtained, carry down a phosphorus complex which by Hammarsten and others is regarded as a lecithin.

2. No method has been found to separate this complex as a whole from the bile salts, as the latter have a tenacious affinity for the phosphorus compound.

3. Bile salts are capable of dissolving and holding in stable solution about 80 per cent. of their weight of egg lecithin, but a considerable portion of this may be separated by precipitation; the amount remaining with the bile salts and not separated by acetone, is much in excess of that probably contained in any bile. It appears likely, therefore, that any method of separation of the two substances must involve the use of reagents much more active than we now have.

4. In the solution of lecithin by bile salts, the addition of inorganic salts hastens the action, but does not increase the amount dissolved. A few inorganic salts, however, precipitate the mixture.

[1] *Loc. cit.*

5. The solution of lecithin in bile salts shows a greatly increased optical rotation, from which it may be calculated that the rotation of the lecithin is about $[\alpha]_D = 7.75°$.

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June, 1908.

NEW BOOKS.

Roscoe and Schorlemmer's Treatise on Chemistry. Volume II.—The Metals. New edition, completely revised by SIR H. E. ROSCOE AND DR. A. HARDEN. New York: The Macmillan Co. 1907. xvi + 1436 pp. Price, $7.50 net.

Roscoe and Schorlemmer's two volumes on inorganic chemistry are in the library of every chemist, and their admirable qualities are so familiar that detailed description is unnecessary. The characteristics which distinguish this work have been preserved in the revised edition and, at the same time, the whole has been brought thoroughly up to date. It is a pleasure to use a book which is so eminently readable, and yet so adequate to its purpose scientifically as is this one. The historical perspective, as heretofore, is carefully maintained throughout, and adds greatly to the charm as well as to the usefulness of the volume. The full and well illustrated accounts of technical processes are still a conspicuous feature of the book. The revision has been most thorough. There are changes in almost every paragraph, and the additions dealing with recent investigations are very numerous and exceedingly well chosen. On the whole, nearly two hundred and fifty pages have been added. In the earlier part of the volume the most noteworthy changes consist in the introduction of sections on Werner's theory of valence, on J. J. Thomson's corpuscular theory of the constitution of the atom, on colloidal solutions of metals, and on the phase rule (in connection with an expanded account of salt-hydrates). The paragraphs on alloys have been enlarged and give an admirable résumé of the subject. The section on crystallography (50 pp.) has been transferred from Volume I to this volume, and has also been revised. A new section on radioactive elements and radioactivity (28 pp.) has been added at the end. The only section which seems to fall conspicuously short of the high level attained by the book as a whole is that on the constitution of steel. This subject is of such great interest, both theoretically and practically, that the very inadequate treatment of recent work is incomprehensible. Roozeboom, Von Jüptner, and Roberts Austen are not even mentioned, although elsewhere references to the latest investigations are always given.

Throughout the book, modern views have been introduced with such skill, and have been incorporated so completely to form a homogeneous product that one is never for a moment reminded of the fact that the first edition was written over thirty years ago. Just as the first edition was